INTERNAL✦MEDICINE

□ CASE REPORT □

Different Effects of Tolvaptan in Patients with Idiopathic Membranous Nephropathy with Nephrotic Syndrome

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Abstract

This case report discusses the clinical indication for immunosuppressants in patients with idiopathic membranous nephropathy (IMN). Because this disease occasionally shows spontaneous remission, it is necessary to determine the predictive values for a therapeutic effect in order to provide appropriate treatment. Two distinct cases described herein illustrate the different effects of tolvaptan in responders and non-responders, according to the pre-treatment levels of AQP-2 immunostaining in the samples from renal biopsy and urinary levels of AQP-2 and osmolality, suggesting that these values may be useful predictors of response to tolvaptan in patients with nephrotic IMN.

Key words: aquaporin-2 (AQP-2), idiopathic membranous nephropathy (IMN), nephrotic syndrome, prednisolone (PSL), tolvaptan

Introduction

Tolvaptan, a vasopressin V2 receptor antagonist, was developed to treat patients with congestive heart failure (1, 2). Tolvaptan decreases body weight, increases the serum sodium levels, and improves congestion and pretibial edema without significantly increasing the rate of adverse events in patients (3). Although the precise mechanism of resistance to tolvaptan is unknown, Imamura et al. reported that aging and chronic kidney disease (CKD) were major risk factors in non-responders (4). Some investigators also reported that non-response to tolvaptan was associated with a decreased renal function (5). Recently, Park et al. reported a patient with minimal change nephrotic syndrome whose refractory edema was controlled immediately by tolvaptan prior to steroid administration (6). However, the effect of tolvaptan in idiopathic membranous nephropathy (IMN) remains uncertain.

Aquaporin (AQP)-2, a protein expressed in the apical membranes of the principal cells of the renal collecting duct, regulates water-absorption and plays a pivotal role in maintaining water-homeostasis (7). Imamura et al. reported the urinary AQP-2 level to be a novel predictor of the response to tolvaptan in patients with decompensated heart failure (1). Urinary AQP-2 levels have been shown to correlate with the pharmacological effect of tolvaptan in CKD patients (8). The urinary AQP-2 level is therefore a predictor of the responsiveness to tolvaptan and a useful marker for AQP-2-guided tolvaptan treatment in such patients (9).

We herein report two cases of IMN with nephrotic syndrome treated with tolvaptan. The two cases illustrate the different effects of tolvaptan in responders and non-responders on AQP-2 immunostaining in kidney biopsy samples, the urinary levels of AQP-2, and urine osmolality. The findings suggest that these parameters are useful for predicting the response to tolvaptan in IMN patients with nephrotic syndrome.

Case Report

Case 1

A 56-year-old Japanese woman was referred because of severe lower-limb edema and general fatigue. She had been healthy prior to admission with no history of renal disease.

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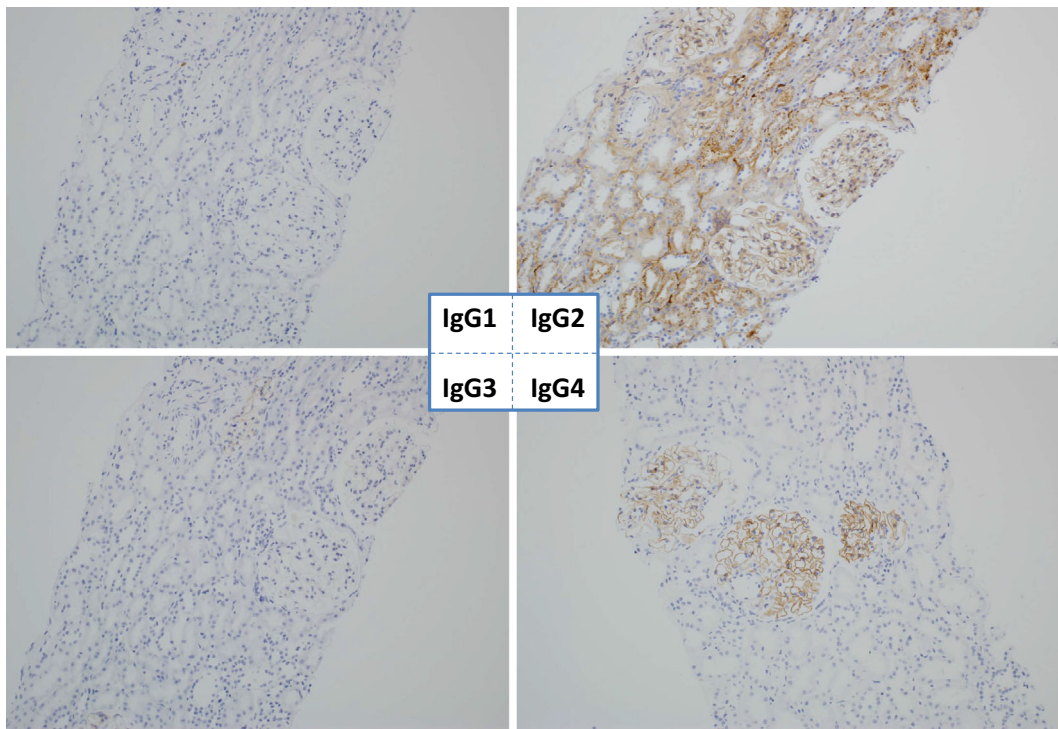
Figure 1. Immunohistochemistry of IgG1, IgG2, IgG3, and IgG4 in Case 1 (magnified 200×). IgG2 and IgG4 were strongly stained in the glomeruli.

One year before admission, laboratory data showed no abnormalities, with a serum creatinine level of 0.68 mg/dL, serum total protein level 7.0 g/mg, low-density lipoprotein (LDL)-cholesterol level 110 mg/dL, and no urinary protein excretion. An examination showed a body height of 152 cm, weight 60 kg (gained about 6 kg in the month after onset), blood pressure (BP) 132/84 mmHg, and heart rate 104 beats/min. Chest and abdominal X-ray showed no abnormalities. The laboratory data revealed severe proteinuria (6.8 g/day), hypoproteinemia (serum total protein 4.2 g/dL, albumin 1.8 g/dL), normal renal function [serum creatinine level 0.66 mg/dL, estimated glomerular filtration ratio (eGFR) 72.2 mL/min/1.73 m^2], and dyslipidemia [LDL-cholesterol level 300 mg/dL, triglyceride (TG) level 220 mg/dL, high-density lipoprotein (HDL)-cholesterol level 56 mg/dL]. Serology was negative for anti-nuclear, anti-glomerular basement membrane, myeloperoxidase anti-neutrophil cytoplasmic (MPO-ANCA), proteinase 3-ANCA, hepatitis C antibodies, and hepatitis B antigen. Serum complement factors and IgG, IgM, IgA levels were within normal limits. Thus, she had no findings suggestive of infection such as hepatitis B and C viruses, malignancy, collagen diseases including systemic lupus erythematosus and rheumatic arthritis, or drug-induced secondary MN. She was therefore diagnosed with IMN.

Despite being treated with conventional diuretics, including a loop diuretic and thiazide (on admission, furosemide 40 mg/day and trichlormethiazide 2 mg/day were administered, and the furosemide dose was increased to 80 mg/day on the 3rd day), her lower-limb edema and urinary volume remained unchanged. On the 5th day, renal biopsy was performed. Histopathology showed typical stage II MN including diffuse thickening of the basement membrane and a large number of electron-condensing substances on the subepithelium (10). An immunofluorescence examination showed granular IgG and C3 deposition on the capillary loops. IgG subclasses were also stained. IgG1, and IgG3 were negative in the glomeruli, but IgG2 and IgG4 were strongly positive (Fig. 1). AQP-2 immunohistochemistry was performed via the following method (polymer technique): The kidney tissues were fixed in 20% neutral buffered formalin and embedded in paraffin, and sections 2-3 μm in thickness were prepared. The details of the method have been reported previously (11). Briefly, the simple statin MAX-PO (MULTI) (NICHIREI BIOSCIENCES INC., Tokyo, Japan) Polymer reagent instillation was performed as follows: incubation at room temperature for 30 minutes, and then washing with TBS for 5 minutes, 3 times. The samples then underwent color development with adjusted DAB substrate solution. The reagent on the slide was washed for a few minutes with purified water and then immersed in purified water. The specimens were finally stained with Hematoxylin and Eosin staining as a contrast agent and then washed for 2 minutes, the color enhanced, and the specimens dehydrated, penetrated, and mounted. The specimens were observed using light microscopy. The primary antibody was anti-AQP-2 antibody (NB110-74682, 1:200, Novus Biologicals, Littleton, Colorado, USA). Case 2 was also stained under the same conditions. AQP-2 immunostaining in the kidney was strongly positive in the collecting duct cells (Fig. 2A). The urinary AQP-2 levels were 3.20 ng/mgCre and urine osmolality 510 mOsm/kgH$_2$O. After the re-
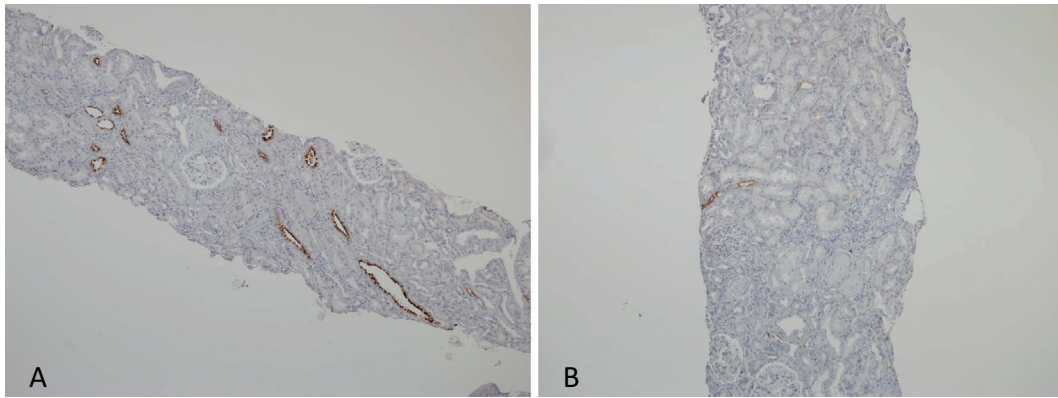
Figure 2. **A: Immunohistochemistry of AQP-2 (brown) in Case 1, a tolvaptan responder (magnified 100×). B: Immunohistochemistry of AQP-2 (brown) in Case 2, a tolvaptan non-responder (magnified 100×). The level of AQP-2 immunostaining was weaker than in Case 1.**
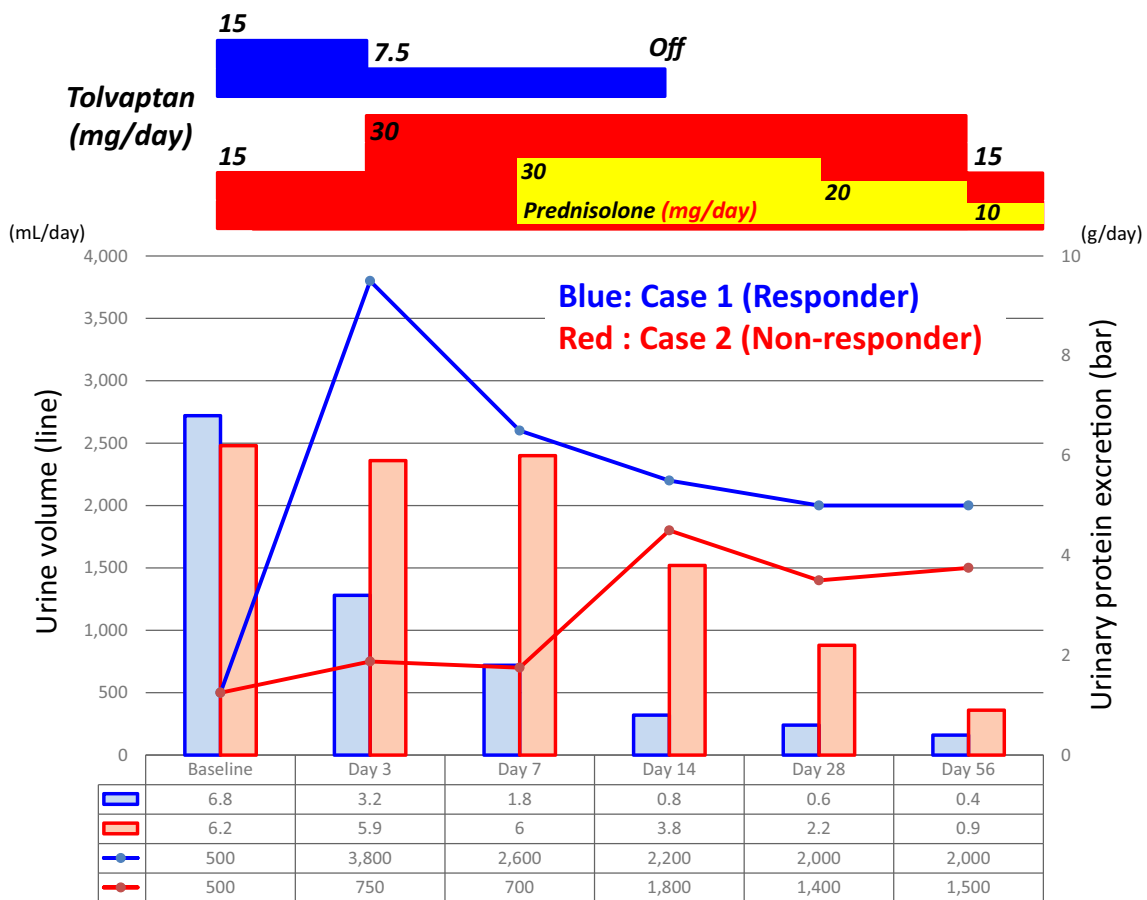

Figure 3. **Clinical course and serial changes in the urine volume and urinary protein excretion in Cases 1 (blue) and 2 (red) after initiation of tolvaptan.**

nal biopsy, tolvaptan 15 mg/day was initiated, and the furosemide dose was reduced to 40 mg/day and trichlormethiazide discontinued due to a marked increase of urine volume. Three days later, the urine volume increased markedly to 3,800 mL/day (Fig. 3, blue line), and tolvaptan was reduced to 7.5 mg/day and then discontinued on the 14th day along with furosemide. The urinary AQP-2 level and urine osmolality also decreased to 0.88 ng/mgCre and 188 mOsm/kgH$_2$O, respectively, on the 14th day. The patient's urine

volume was maintained at approximately 2,000 mL/day by the 56th day. The urinary protein excretion decreased in a serial manner without the administration of an immunosuppressant such as prednisolone (PSL) and had reduced to approximately 0.5 g/day by the 56th day (Fig. 3, blue bar).

The changes in the serum sodium level are shown in Fig. 4. Serum BNP levels were reduced from 250 to 44 pg/mL after tolvaptan treatment on the 14th day. The cardiac function measured by echocardiography remained un-
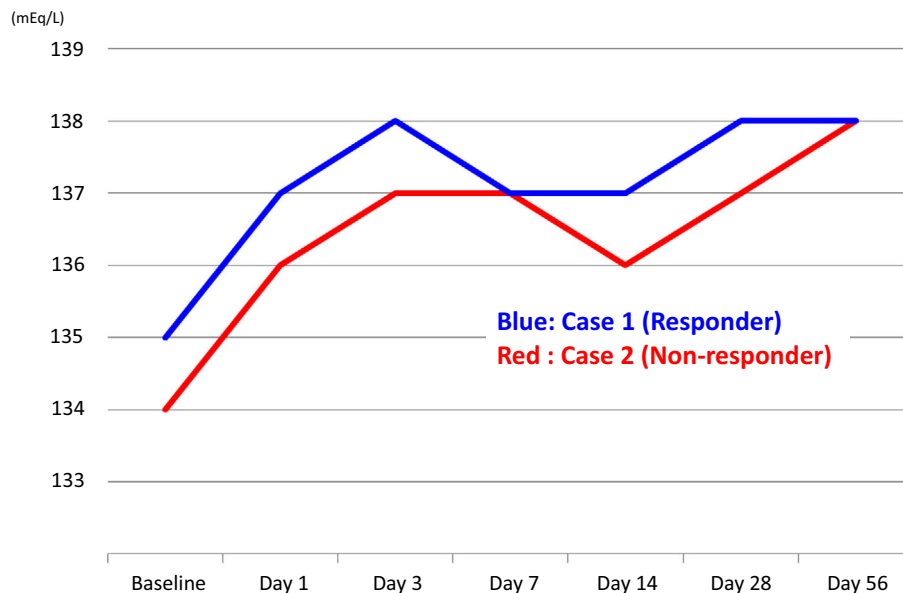
Figure 4. Changes in the serum sodium levels in Case 1 (tolvaptan responder) and Case 2 (tolvaptan non-responder). There was no excess increase in the serum sodium levels in either case.

changed within normal limits throughout the treatment period [baseline: left ventricular end-diastolic dimension (LVDd) 48 mm, left ventricular ejection fraction (LVEF) 66%, E/e' 6.6; 14th day: LVDd 47 mm, LVEF 68%, E/e' 6.5].

Case 2

A 50-year-old Japanese woman was referred because of appetite loss and severe lower-limb edema. She had been healthy with no abnormalities until admission. Six months before admission, her serum creatinine was 0.65 mg/dL with no urinary protein excretion. An examination revealed a body height of 154 cm, weight 61 kg (gained about 7 kg in the month after onset), BP 130/80 mmHg, and heart rate 98 beats/min. Chest and abdominal X-ray showed no abnormalities. The laboratory data showed severe proteinuria (6.2 g/day), hypoproteinemia (serum total protein level 4.3 g/dL, albumin level 2.0 g/dL), dyslipidemia (LDL-cholesterol level 280 mg/dL, TG level 218 mg/dL, HDL-cholesterol level 62 mg/dL), but normal renal function (serum creatinine level 0.68 mg/dL, eGFR 71.2 mL/min/1.73 m^2). Since the serologies and history of causal disease were all negative, as in Case 1, she was also diagnosed with IMN.

Despite being treated with conventional diuretics (on admission furosemide 40 mg/day and trichlormethiazide 2 mg/day were administered, and on the 3rd day, the furosemide and trichlormethiazide doses were increased to 80 and 4 mg/day, respectively), her lower-limb edema remained unchanged. On the 6th day, renal biopsy was performed, and histopathology showed typical stage II MN with severity almost the same as that observed in Case 1. IgG1 and IgG3 were negative in the glomeruli, but IgG2 and IgG4 were positive, showing almost the same staining pattern as Case 1. The expression of AQP-2 was lower than in Case 1 (Fig. 2B). The urinary AQP-2 level and urine osmolality

were also lower (0.86 ng/mgCre and 168 mOsm/kgH$_2$O, respectively) than those measured in Case 1. Tolvaptan 15 mg/day was administered after renal biopsy, resulting in only a small increase in the urine volume. The dose of tolvaptan was increased to 30 mg/day 3 days after initiation. However, the urine volume and protein excretion showed little change over the next 7 days (Fig. 3, red line). PSL (30 mg/day) was then administered, leading to a decrease in the urinary protein excretion to 3.8 g/day by the 14th day. However, both the urinary AQP-2 level and osmolality remained low (0.82 ng/mgCre and 160 mOsm/kgH$_2$O, respectively). After the initiation of PSL, the urine volume increased to 1,800 mL/day by the 14th day and remained at that level until the 56th day (Fig. 3, red line). The urinary protein excretion also decreased to 0.9 g/day by the 56th day (Fig. 3, red bar). The doses of tolvaptan and PSL were then decreased to 15 and 10 mg/day, respectively. In addition, the doses of furosemide and trichlormethiazide were decreased to 40 and 2 mg/day on the 28th day, respectively, and trichlormethiazide was discontinued on the 56th day.

The changes in the serum sodium level are shown in Fig. 4. Serum BNP levels were reduced from 238 to 128 pg/mL on the 14th day and to 56 pg/mL on the 28th day. The cardiac function measured by echocardiography remained unchanged (baseline: LVDd 50 mm, LVEF 68%, E/e' 6.5; 14th day: LVDd 49 mm, LVEF 68%, E/e' 6.4).

The present treatment protocol was conducted in accordance with the Declaration of Helsinki, and the use of tolvaptan, immunostaining of AQP-2, and urinary AQP-2 measurement in the 5 IMN patients with nephrotic syndrome were approved by the Ethics Committee of Shinmatsudo Central General Hospital. Written informed consent was obtained from both patients.

Discussion

IMN occurs typically in middle-aged adults, with the main clinical manifestation being the nephrotic syndrome (10). Treatment of IMN using immunosuppressants is controversial, because there are two conflicting possibilities: 1) progression to renal failure and end-stage renal disease, 2) spontaneous remission, which is more common than expected (12, 13). Although it is difficult to predict the best treatment for IMN, it is ideal to distinguish between patients and delay intensive treatment in cases of spontaneous remission. Immunosuppressants, including PSL, were not administered in Case 1, due in part, to the effectiveness of tolvaptan, whereas PSL was needed in Case 2 because the patient did not respond to tolvaptan.

Tolvaptan promotes water clearance without causing a deterioration in serum electrolyte levels in patients with congestive heart failure. In general, expression of AQP-2 decreases in patients with CKD (8). We have reported that AQP-2 immunostaining is absent in renal tissues of tolvaptan non-responders with diabetic nephropathy but expressed strongly in responders (11). In the present cases, AQP-2 was expressed strongly in the renal tissue of the tolvaptan responder (Case 1) but only weakly in the non-responder (Case 2). In the present cases, urinary AQP-2 concentration and urine osmolality were also higher in the responder than in the non-responder. Imamura et al. have reported that urine AQP-2 is a novel predictor for identifying responders to tolvaptan, especially in patients with heart failure (1, 2). Iwatani et al. also reported that a urine osmolality cut-off point of 279 mOsm/kgH$_2$O predicted the reduction in body weight associated with tolvaptan in patients with CKD (14). In the present report, the urine osmolality was 510 mOsm/kgH$_2$O in the responder to tolvaptan and 168 mOsm/kgH$_2$O in the non-responder. Interestingly, in the non-responder, the urine volume increased significantly after the initiation of PSL therapy. Chen et al. reported the findings from an *ex vivo* study using suspensions of kidney inner medullary collecting duct cells that showed that dexamethasone directly upregulated the expression of cellular AQP-2. This suggested that dexamethasone may improve the responsiveness to tolvaptan by increasing AQP-2 expression (7). Therefore, there may be additional and favorable effects of PSL treatment for non-responders to tolvaptan, such as in Case 2. Taken together, these present and previous findings suggest that evaluating these markers may be useful for identifying tolvaptan responders in nephrotic IMN patients and predict their need for additional treatment, such as with immunosuppressants.

Because the cardiac function, volume overload, and the use of diuretics have been reported to alter the expression of AQP-2, the association between these parameters and AQP-2 expression is important. In the present cases, although severe pretibial edema due to nephrotic syndrome existed, we found no congestive heart failure. In the present cases, since we did not perform right heart catheterization, we could not obtain the precise hemodynamic parameters, such as mean pulmonary capillary wedge pressure, mean right atrial pressure, and systolic pulmonary artery pressure. The serum BNP levels were indeed higher than normal values, but the cardiac function was within normal limits throughout the treatment period. Moreover, the doses of diuretics were comparable between the present cases prior to the initiation of tovlaptan. Taken together, these findings suggest that urinary AQP-2 and kidney AQP-2 expression may be useful markers in patients with nephrotic syndrome without clinical heart failure.

It is important to examine the relationship between the urinary AQP-2 levels and pathological AQP-2 staining. However, to our knowledge, little is known about this relationship. In a preliminary case report, we reported that AQP-2 immunostaining and urinary AQP-2 concentrations were important predictors of response to tolvaptan in diabetic nephrotic syndrome patients with congestive heart failure (15). The amount of AQP-2 at steady state represents a balance between the production of AQP-2 by translation and removal from the cells by either degradation or exosome secretion (16). Changes in the rate of AQP-2 production can be due to changes in the abundance of AQP-2 mRNA or to direct regulation of translation (16). An increase in urinary AQP-2 excretion could reflect either an increase in synthesis or a decrease in degradation. More examinations using molecular biological methods, such as *in situ* hybridization and polymerase chain reaction, will be required in the future. In addition, it is still unclear whether or not there is a relationship between the effectiveness of tolvaptan and the type of MN - idiopathic or secondary MN. Thus, the presence of a relationship between the effectiveness of tolvaptan and the type of MN should be evaluated.

In summary, we report for the first time the different effects of tolvaptan in IMN patients with nephrotic syndrome. The renal AQP-2 expression, urinary AQP-2 concentration, and urine osmolality may be important predictors for distinguishing between responders and non-responders to tolvaptan in patients with IMN and nephrotic syndrome.

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