Urine IgM excretion predicts outcome in ANCA-associated renal vasculitis

Omran Bakoush¹, Märten Segelmark¹, Ole Torffvit², Sophie Ohlsson¹ and Jan Tencer²

¹Department of Nephrology and ²Department of Medicine, Lund University Hospital, Sweden

Abstract

Background. Renal function at diagnosis is a strong predictor not only of renal survival but also of patient survival of those with anti-neutrophil cytoplasmic antibody (ANCA)-associated small vessel vasculitis (ASVV). Apart from the renal function at diagnosis, there are no other established risk factors for renal outcome in ASVV. We have previously reported that in other forms of glomerular diseases, an increased urine excretion of IgM is an early marker of poor renal outcome.

Methods. In this single-centre observational study, the prognostic significance of urine IgM excretion and other selected prognostic markers was studied in 83 consecutive patients (49 males, 34 females) with ASVV with renal involvement.

Results. Patient survival at 1 and 5 years was 93 and 77%, respectively, and the corresponding figures for renal survival censored for death were 84 and 76%. Univariate analysis indicated that patient survival was inversely associated with age, male sex, serum creatinine, low serum albumin and high urine IgM excretion. Renal survival was inversely associated with serum creatinine, albuminuria and urine IgM. Multivariate analysis determined that only old age and high urine IgM excretion were independent predictors of patient survival [odds ratio (OR) = 11.2 and 4.4, respectively, \( P < 0.01 \)]. Urine excretion of IgM was the only independent predictor of end-stage renal disease (OR = 19.8, \( P = 0.004 \)). Overall, 35% of the patients reached the composite end-point of either death or renal replacement therapy. Urine IgM excretion was the most potent single predictor of such an outcome (OR = 7.7, \( P = 0.000 \)).

Conclusion. The occurrence of an increased amount of IgM in urine at presentation is a strong marker of poor prognosis for patients with ANCA-associated renal vasculitis.

Keywords: ANCA; glomerulonephritis; IgM; microscopic polyangiitis; vasculitis; Wegener’s granulomatosis

Introduction

Small vessel vasculitides, such as Wegener’s granulomatosis and microscopic polyangiitis, are strongly associated with anti-neutrophil cytoplasmic antibodies (ANCAs), directed to either myeloperoxidase (MPO) or proteinase 3 (PR3) [1]. ANCA-associated small vessel vasculitis (ASVV) is a group of multisystem disorders with an annual incidence between 9.5 and 16/million/year [2]. They are characterized by a high incidence of renal involvement; figures between 80 and 94% have been reported [1,3,4]. Since the introduction of cyclophosphamide and prednisolone as the standard treatment of ASVV, the patient survival rate has improved dramatically from <20% at 1 year to at least 60% at 5 years [1,3–6]. However, mortality is substantial and morbidity remains high; many patients develop end-stage renal disease (ESRD) [6]. Previous studies have shown that the renal function at diagnosis is a strong predictor not only of renal survival but also of patient survival in ASVV [7–9]. Other factors have also been reported to predict the outcome in ASVV, such as severity of the disease at diagnosis, treatment-related infections, \( ß \)-antitrypsin deficiency, high levels of PR3-ANCA measured by capture enzyme-linked immunosorbent assay (ELISA) and low levels of thrombocytes [6,10–13]. However, these findings have usually not been confirmed in repeated investigations. Proteinuria, severe interstitial fibrosis and glomerulosclerosis, which are known to predict the outcome in chronic proteinuric glomerulonephritides, have also been found to be important risk factors for the development of renal failure in ASVV [7,9,14,15].

In other glomerulonephritides, not associated with vasculitis, elevated urine excretion of high molecular weight proteins, e.g. IgM, has been found to be a better
predictor of renal outcome than the degree of albuminuria [16–18]. Tencer et al. reported high urine IgM excretion in many patients with glomerulonephritis and ASVV [19]. However, the prognostic value of urine IgM excretion has not been studied in these patients. The aim of this study was to investigate the prognostic significance of urine IgM excretion in ASVV compared with other known or putative prognostic markers.

Subjects and methods

Patients

The patients in this study were all participants in a long-term prospective investigation programme of glomerular diseases conducted at the Department of Nephrology, University Hospital of Lund, Sweden. Results from this programme, including earlier cohorts of ASVV, have previously been published from our institution [4,6,17,19,20]. In the present study, patients with ANCA-associated pauci-immune glomerulonephritis, diagnosed between June 1993 and September 2002, were included. The morphological diagnoses of the disease were established by evaluation of representative percutaneous renal biopsy specimens using light microscopy and immunofluorescence staining. Patients with other small vessel vasculitides, such as Goodpasture’s disease, or with other histological diagnosis, such as post-infectious glomerulonephritis, diabetic nephropathy, nephrosclerosis and IgA nephropathy, were excluded. Renal biopsy was not performed in four patients. These patients had histological verification of Wegener’s granulomatosis by specimens obtained from the respiratory tract in conjunction with clinical signs of rapidly progressive glomerulonephritis. The study was approved by the local ethics committee.

Clinical diagnosis, treatment and follow-up

The patients were subclassified into Wegener’s granulomatosis and microscopic polyangiitis according to the Chapel-Hill consensus conference. Only patients with granulomatous diseases, either histology proven or strongly indicated by non-invasive diagnostic procedures, were classified as Wegener’s granulomatosis. The patients were treated with oral cyclophosphamide, 2 mg/kg per day, and prednisolone 1 mg/kg per day. Adjunctive therapy with plasmapheresis or pulse methylprednisolone was given to 29 patients with severe renal disease or renopulmonary syndrome. The prednisolone dosage was tapered to 15–20 mg daily at 3 months after initiation. After approximately 6 months of remission, a switch was usually made from cyclophosphamide to azathioprine, 1–2 mg/kg per day orally. Six patients received induction treatment with oral azathioprine 2 mg/kg, and one patient with methotrexate. Immunosuppressive treatment was withheld in one case because of good response to immunoglobulin treatment, and three patients were treated only with oral prednisolone.

All the patients were followed on a regular basis at the nephrology out-patient clinics and were on a normal protein diet. The patients were followed for up to 60 months or until September 2004. The number of patients, age, gender and their baseline data are presented in Table 1. The primary end-point was death during the first 5 years of the disease, and the secondary end-point was the development of ESRD during the same time. For survival analysis, the patients were divided into subgroups of equal size according to the median level of the selected variables at presentation. Survival time was calculated from the date of diagnosis.

Laboratory analysis

The blood samples and the first voided urine specimens were obtained on the morning of the day the kidney biopsy was performed. Portions of 30 ml of urine were collected in polyethylene vessels (Kebo AB, Sweden). The samples, after addition of 1 ml of a preservation solution, were kept frozen at −20 °C until assayed. The preservation solution described earlier contained benzamidinium chloride, EDTA, sodium azide and Tris base, representing inhibitors of serine- and metalloproteinase, an antimicrobial agent and a buffer substance, respectively. This solution has previously been demonstrated to result in stable levels of proteins in frozen urine samples [21]. Serum and urine creatinine were

<table>
<thead>
<tr>
<th>Variable</th>
<th>WG group</th>
<th>MPA group</th>
<th>All patients</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
<td>46</td>
<td>83</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female</td>
<td>21/16</td>
<td>28/18</td>
<td>49/34</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>59 (18–75)</td>
<td>69 (39–82)</td>
<td>65</td>
<td>0.002</td>
</tr>
<tr>
<td>BVAS</td>
<td>21 (6–32)</td>
<td>16 (6–27)</td>
<td>18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PR3-ANCA positive</td>
<td>29</td>
<td>11</td>
<td>40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MPO-ANCA positive</td>
<td>8</td>
<td>35</td>
<td>43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>37 (5–249)</td>
<td>32 (5–218)</td>
<td>37</td>
<td>NS</td>
</tr>
<tr>
<td>Thrombocytes (×10³/l)</td>
<td>294 (138–1091)</td>
<td>327 (73–726)</td>
<td>322</td>
<td>NS</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>30 (13–45)</td>
<td>29 (18–44)</td>
<td>29</td>
<td>NS</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>173 (44–859)</td>
<td>296 (74–1716)</td>
<td>297</td>
<td>0.015</td>
</tr>
<tr>
<td>Creatinine clearance</td>
<td>35 (6–164)</td>
<td>18 (1–108)</td>
<td>28</td>
<td>0.004</td>
</tr>
<tr>
<td>Dialysis at diagnosis</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>NS</td>
</tr>
<tr>
<td>Albuminuria (mg/mmol)</td>
<td>24 (0.12–1238)</td>
<td>81 (0.7–815)</td>
<td>50</td>
<td>0.016</td>
</tr>
<tr>
<td>IgM-uria (mg/mmol)</td>
<td>0.03 (0.01–0.97)</td>
<td>0.06 (0.01–2.0)</td>
<td>0.05</td>
<td>0.024</td>
</tr>
</tbody>
</table>

CRP = C-reactive protein.
BUAS = Birmingham Vasculitis Activity Score.
determined enzymatically using a Kodak Ektachem 700 XRC system. Serum and urine albumin were determined by immunoturbidimetry using a Cobas Mira S system (Roche Inc., Stockholm, Sweden) and monospecific rabbit antisera obtained from Duko (Copenhagen, Denmark). Urine IgM was measured by an ELISA technique described in detail elsewhere. The lower detection limit for the urine IgM assay is 1 mg/l; the intra-assay and inter-assay variation is 4.6 and 10.9%, respectively [19] (Figure 1). We used the urine protein to creatinine ratio measured in a spot urine sample since, from our experience and that of many others, it is a reliable estimate of the degree of proteinuria and is highly correlated with 24 h urine protein excretion [22]. Furthermore, it helps to avoid errors caused by problems during urine sampling. The urine IgM creatinine index and fractional clearance of urine IgM were highly correlated ($r = 0.9$, $P < 0.001$) in the studied patients. Creatinine clearance ($C_{cr}$) was calculated using the Cockcroft and Gault formula [23] where: $C_{cr} = [88 \times (145 - \text{age})/\text{serum creatinine}] - 3$ (15% lower for women).

Serum ANCA analysis was performed by ELISA using microtiter plates coated with isolated human PR3 and with human granulocyte MPO obtained from Wieslab AB (Lund Sweden). The assays were performed as described elsewhere [4].

Morphological diagnoses were established by renal biopsies, analysed by light microscopy at the Department of Pathology, University Hospital of Lund. The biopsies showed extra-capillary or necrotizing glomerulonephritis in at least one glomerulus. Immunofluorescence staining showed no or only minor deposits of immunocomplexes (pauci-immune). Semi-quantitative scoring of glomerular and interstitial injury was done in a blind manner. The degree of interstitial fibrosis was scored as 0 for normal interstitium, 1 for mild and 2 for a moderate to severe degree of interstitial fibrosis. The percentage of biopsies with necrosis, the percentage of the glomeruli with crescent formation and the percentage of glomeruli with global glomerulosclerosis in the biopsy specimen were calculated. Glomeruli free of crescent formation or glomerulosclerotic changes were regarded as normal.

**Statistical method of analysis**

Statistical analysis was performed with SPSS 12.0.1 for Windows (SPSS Inc., Chicago, IL). The patients were divided into equal groups according to the median of selected variables. The difference between subgroups was compared by the non-parametric, Mann–Whitney U-test. $P$-values $<0.05$ were considered to be significant. Renal (ESRD-free) survival and patient survival were described using the Kaplan–Meier method. Cox’s proportional hazards regression analysis was used to investigate whether selected variables predicted renal or patient survival. Factors that did not affect survival significantly were removed by a stepwise procedure according to a likelihood ratio.

**Results**

Eighty-three (83) patients (49 males and 34 females) were included in the study (Table 1). The median age was 65 years (range 18–82), median serum albumin was 29 g/l (13–45), median serum creatinine was 257 $\mu$mol/l (44–1716) and median urine albumin excretion was 50 mg/mmol creatinine (0.12–1238). The median urine concentration of IgM was 0.05 mg/mmol creatinine (0.01–2).

The patients were followed-up for up to 60 months after diagnosis. All the patients completed a 5-year follow-up, except those who died (19 patients) or those included after September 1999 (12 patients). No patient was lost to follow-up. Patients with MPO-ANCA were older than those with PR3-ANCA (68 and 59 years, respectively, $P = 0.055$) and had significantly higher serum creatinine levels (295 and 155, respectively, $P = 0.013$). The male patients presented with significantly higher serum creatinine levels than the females (370 and 262, respectively, $P = 0.006$). However, the age at onset of the disease was not significantly different (mean 63 and 58 years, respectively).

**Patient survival**

A total of 19 patients died during the follow-up time (Table 2). The overall 1- and 5-year patient survival rates were 93 and 77%, respectively. Six patients

<table>
<thead>
<tr>
<th>Causes of death</th>
<th>n (%)</th>
<th>Within 1 year</th>
<th>After 1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular death</td>
<td>9 (47%)</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Severe vasculitis</td>
<td>3 (15.8%)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td>5 (26%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brain abscess (nocardia)</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Sepsis</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Chronic respiratory failure</td>
<td>2</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>6</td>
<td>13</td>
</tr>
</tbody>
</table>
died during the first year. Three of them succumbed to severe vasculitic diseases with involvement of other major organs, e.g., lung or brain, two patients died due to cardiovascular events, and one patient died due to infection. Late mortality was mainly due to cardiovascular events (seven patients), while four patients died from infectious complications, and two from chronic respiratory disease (Table 2).

Univariate analysis revealed that the relative risk of death was three times higher in the male patients [odds ratio (OR) = 3, \( P = 0.05 \)]. It was also significantly higher in patients older than 65 years (OR = 11.2, \( P = 0.001 \)) (Figure 2), with serum albumin < 30 g/l (OR = 8.9, \( P = 0.003 \)), in those with serum creatinine > 250 \( \mu \)mol/l at diagnosis (OR = 3.4, \( P = 0.018 \)) or \( C_{cr} \leq 28 \text{ml/min} \) (OR = 3.4, \( P < 0.05 \)), high urine albumin excretion (OR = 10.4, \( P = 0.002 \)) and high urine IgM excretion (OR = 19.8, \( P = 0.004 \)). Age, sex, serum albumin, CRP, thrombocytes, ANCA levels, ANCA type (PR3/MPO) and the diagnosis were not associated with impaired renal survival. Multivariate analysis, using Cox’s regression, showed that only high urine IgM excretion was an independent predictor of renal survival (\( P = 0.03 \)).

Patients with high urine IgM excretion had a renal survival rate at 1 year of 69\%, and a 5-year renal survival rate of 59\%. Patients with low urine IgM excretion had an excellent renal survival rate of 97\% at both 1 and 5 years after diagnosis. The only patient with low urine IgM excretion at diagnosis who was on renal replacement therapy at follow-up was already dialysis dependent at presentation. No other patient in this group developed ESRD during the follow-up. In contrast, five out of 41 patients with serum creatinine > 250 \( \mu \)mol/l at diagnosis and high urine IgM progressed to ESRD during the same time.

A semi-quantitative evaluation of histological lesions showed that in patients with high urine IgM excretion, a mean of 37\% of the glomeruli in the kidney biopsies were histologically normal and 43\% had crescent formation compared with 70\% who were normal and 19\% who had crescent formation in patients with low urine IgM excretion (\( P < 0.005 \)). Necrotizing glomerular lesions were seen in 76\% of the biopsies in patients with high urine IgM excretion compared with 53\% in the low urine IgM excretion group (\( P = 0.026 \)).

Patients with high urine IgM excretion had significantly lower serum albumin levels (27 and 31, respectively, \( P = 0.006 \)) and a tendency towards higher CRP (82 and 52, respectively, \( P = 0.06 \)) and serum IgM (0.9 and 0.7, respectively, \( P = 0.06 \)) levels as compared with patients with low IgM excretion.
Serum albumin showed no significant association with the degree of albuminuria ($r = 0.16$, NS).

The average Birmingham vasculitis activity score (BVAS) at presentation of studied patients was 18.3. It was significantly higher in patients with Wegener’s granulomatosis compared with those with microscopic polyangiitis (Table 1). BVAS did not predict prognosis in the studied patients. There is no correlation between urine IgM excretion and degree of BVAS. BVAS in patients with low and high IgM groups was 17.2 and 19.3, respectively ($P=NS$). High urine IgM excretion was an independent predictor of poor patient and renal survival both in patients with Wegener’s granulomatosis and in those with microscopic polyangiitis.

Combined end-point (death with native kidney function or ESRD)

A total of 29 patients (16 of them at 1 year) developed ESRD or died with functioning native kidneys during the 5 years of follow-up. The overall incidence of the composite end-point was 18% at 1 year and 35% at 5 years. The composite end-points, ESRD or death, were significantly associated with positive serology for MPO-ANCA ($OR = 2.4$, $P = 0.034$), low serum albumin ($OR = 2.8$, $P = 0.013$), high serum creatinine ($OR = 2.9$, $P = 0.008$), high urine albumin excretion ($OR = 3.9$, $P = 0.002$) and high urine IgM excretion ($OR = 7.7$, $P = 0.000$). No significant association was found with age or gender. With multivariable Cox’s regression analysis, only urine IgM excretion remained a significant independent predictor of the composite end-points ($OR = 4.8$, $P = 0.009$, Figure 3). These end-points were only reached by 3% at 1 year and 11.2% at 5 years among the patients with low urine IgM excretion, compared with 35% at 1 year and 56% at 5 years among the patients with high urine IgM excretion.

Discussion

The present study evaluates patient and renal survival in a single-centre cohort consisting of 83 patients with ASVV and renal involvement at 1 and 5 years after diagnosis. Patient survival was similar to previous studies of patients with the same diagnosis [6,8,9,12,24,25]. However, compared with a previous report from our centre of a study by Westman et al. including patients from 1971 up to 1993, this present report shows a slight improvement in 1 and 5 year mortality of 8 and 4%, respectively, although the median age of the cohort was 3 years older [6]. This is the first study to report the prognostic significance of urine IgM excretion in these diseases. The study indicates that urinary IgM is a better independent predictor of renal survival than serum creatinine at diagnosis and, in addition to age, it is an important predictor for patient survival. In this cohort, 56% of the patients with high urine IgM excretion, compared with only 11% of the patients with low urine IgM excretion, either died with functioning native kidneys or developed ESRD at the 5-year follow-up (Figure 3).

The poor renal survival we found in ASVV patients with high urine IgM excretion is in accordance with our previous findings regarding patients with non-vasculitic glomerular diseases [17,18]. In these latter studies, we found that patients with low urine IgM excretion maintained their kidney function despite a high degree of albuminuria, and patients with high urine IgM excretion were more likely to develop ESRD.

Very large proteins, such as IgM (molecular radius 120 Å), and red blood cells are able to pass the glomerular capillary wall (GCW) only through the large defects (shunts) in the GCW. The intact GCW exhibits very few of these, and a repairing apparatus normally seals these shunts. Varying degrees of ultrastructural defects in glomerular basement membranes, measuring between 15 and 200 nm in diameter, were revealed in glomerulonephritis patients by transmission electron microscopy using a tissue negative staining method. These defects are not seen in normal renal tissue [26]. Thus, the occurrence of IgM in the final urine reflects a markedly increased population of highly unselective pathways, shunts and large defects in the GCW, thereby reflecting the severity of glomerular damage [17,19,27,28].

Also, in this study, kidney biopsies of the patients with high urine IgM excretion revealed a significantly high percentage of glomerular crescent formation and the presence of necrotizing glomerular lesions, and were more likely to have severe interstitial fibrosis. Thus, proteins the size of IgM could make a sensitive marker of such lesions, which might explain the correlation between the degree of urinary IgM excretion in
glomerular diseases, the tubulointerstitial fibrosis and the decline in renal function.

The proteinuria selectivity index was previously used to distinguish between nephrotic patients with mostly albuminuria and those with increased excretion of high molecular weight proteins as well. Patients with vasculitis seldom have nephrotic range proteinuria but often have increased urine excretion of high molecular weight proteins. A low proteinuria selectivity index, i.e. proteinuria with a high percentage of high molecular weight proteins, is a poor prognostic marker in vasculitic patients.

Atherosclerotic vascular diseases, common in elderly patients and patients with metabolic syndrome, may cause an increase in urinary excretion of high molecular weight proteins, such as IgM, because of ischaemic glomerulosclerosis [29]. Superimposed vasculitic renal disease in elderly patients with many sclerotic glomeruli and a low reserve of normally functioning nephrons will be associated with severe renal failure and poor outcome [30,31]. However, in this study, the percentage of global glomerulosclerosis in low and high urine IgM groups was almost identical, implying that the increased urine IgM excretion in the studied patients was mainly due to severe glomerulonephritis. We believe that urine IgM excretion is a marker of acute ongoing damage, while serum creatinine is more reflective of the cumulative glomerular damage.

The type and duration of immunosuppressive treatment was given regardless of urine IgM concentration. However, 50% of the patients with high urine IgM excretion received adjunctive therapy with plasmapheresis compared with only 18% of patients in the low IgM group. We found it unlikely that this difference would contribute to the adverse outcome of the high IgM group.

The urine IgM assay is a simple, easy technique and can be incorporated in the routine laboratory analysis in follow-up of patients with AASV.

In accordance with other reports, this study demonstrated that mortality was strongly associated with old age and was mostly due to cardiovascular deaths, severe vasculitis and severe infectious complications [6,8,25,32–34]. As early as during the first year of the follow-up, older patients had a very steep mortality rate (15%), which can be compared with null mortality in younger patients. This finding clearly highlights the dilemma that the clinician treating these patients must face, and it emphasizes that elderly patients follow a different clinical course with a high rate of mortality [32].

The serum creatinine level is reported to be the strongest predictor of outcome in vasculitic patients [3,8,32]. Serum creatinine seems to be a stronger predictor for mortality only when it reaches a level where dialysis treatment is required, i.e. cases of severe renal impairment [6,12,24,32,35,36]. In the present cohort, increased urine IgM excretion appeared to be an early predictor, before the stage of dialysis requirement, of a high risk of mortality and progression to ESRD.

As described previously in vasculitic and non-vasculitic glomerulonephritis, this study demonstrates that the male patients had poorer outcome than the female patients and mortality was high in patients with a low serum albumin level [12,32]. The male patients tended to delay seeking medical help for an already severe renal impairment. Low serum albumin probably reflects the severity of vasculitic illness, as there was no association between the serum albumin level and the degree of albuminuria.

In this study, patients with microscopic polyangiitis had a poorer outcome than those with Wegener’s granulomatosis, as they were older and had a more advanced renal disease at presentation (Table 1). We were unable to find any significant correlation between patient outcome and plasma levels of PR3- or MPO-ANCA as described elsewhere, possibly because of the small size of our cohort [3,4,6,13,35].

In conclusion, for patients with ANCA-associated small vessel vasculitis, a high level of urine IgM excretion is strongly associated with the development of ESRD and, in addition to old age, also predicts patient survival. We recommend routine measurement of urine IgM concentrations at diagnosis in patients with ANCA-associated small vessel vasculitis as a relatively cheap, non-invasive and early prognostic marker.

Further studies are needed to evaluate the measurement of urine IgM during the course of the disease for early identification of patients who are likely to relapse. Studies are also needed to validate these results in other types of vasculitic renal diseases, such as systemic lupus erythematosus nephritis, and to evaluate the association between urine IgM excretion and excretion of other biologically active proteins, such as transforming growth factor-β and monocyte chemotactic protein-1 to illustrate further mechanisms of vasculitic renal disease.

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