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BRIEF REPORT

**María del Carmen Laso · María Estela Cadario · Laura Haymes · Irene Grimoldi · Ziomara Balbarrey · Enrique V. Casanueva**

# *Mycoplasma pneumoniae* detection with PCR in renal tissue of a patient with acute glomerulonephritis

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**Abstract** Renal disease concurrent with a *Mycoplasma pneumoniae* infection is uncommon. In this report we describe the clinical outcome of a 6-year-old patient who presented with a rapidly progressive glomerulonephritis that required dialysis. A kidney biopsy was performed, and the results revealed membranoprolipherative glomerulonephritis. The IgM serology was positive, and *M. pneumoniae* DNA was detected in a renal biopsy sample using a nested-PCR assay. The outcome was good.

**Keywords** Glomerulonephritis · Mycoplasma pneumoniae · Polymerase chain reaction

## Introduction

Acute infectious glomerulonephritis is associated with infection by several bacteria, viruses and parasites [1, 2], the most common being infections caused by Group A β-hemolytic *Streptococcus*; staphylococcal, pneumococcal and mycoplasmal infections are less common causes [1].

*Mycoplasma pneumoniae* typically is an etiologic agent of human respiratory infections. However, extrapulmonar complications have been reported at different times during the course of respiratory infections [3]. The most common extrapulmonar signs are those related with the central nervous system and the skin, while acute glomerulonephritis, renal failure, tubulo-interstitial nephritis and IgA nephropathy have been only rarely described in association with *M. pneumoniae* infections [3].

It is very difficult to find a direct relationship between infections and renal inflammatory lesions, and a literature search revealed that there are very few reports of the antigen appearing in renal tissue [4–6].

We report a 6-year old child who presented with a rapidly progressive glomerulonephritis and in whom *M. pneumoniae* DNA was detected in a renal biopsy sample using a nested-PCR assay [7].

## Case report

A 6-year-old boy was admitted to our hospital. He reported oliguria during the previous 24 h in association with fever and vomiting. Two days before he had attended with fever; penicillin V had been prescribed because of a congestive throat. At the time of admission he had only received three doses. Physical examination showed a normohydrated, febrile boy, with a generalized non-pruriginous morbiliform exanthema that predominated in the trunk and face. Bilateral parotid swelling, lateral cervical adenopathies, diffuse abdominal pain and hepatosplenomegaly were also found.

The patient presented with slight periorbital and pretibial edema.

The patient, his father and two siblings had thalassemia. His blood pressure was 99/64 mmHg. Laboratory evaluation at admission included hematocrit, 25%; hemoglobin, 8 g/l; white blood cells, 11.5×10/l; platelets, 183×10/l; urea, 160 mg/dl; creatinine, 3.61 mg/dl (kinetic method); sodium, 143 mEq/l; potassium, 5.8 mEq/l; antistreptolysin O

M. del Carmen Laso (✉) · L. Haymes · I. Grimoldi
Nephrology Department, Hospital Municipal del Niño,
Granada 4175 (1754),
San Justo, Buenos Aires Province, Argentina
e-mail: mlaso@intramed.net.ar
Tel.: +54-4441-9371
Fax: +54-4441-9371

M. E. Cadario
Virology Department, Service of Respiratory Viral Diseases,
INEI-ANLIS "Dr. Carlos Malbrán",
Buenos Aires, Argentina

Z. Balbarrey
Pathology Department,
Hospital Nacional de Pediatría "Dr. Juan P. Garrahan",
Buenos Aires, Argentina

E. V. Casanueva
Infectology Unit, Hospital Municipal del Niño,
San Justo, Buenos Aires Province, Argentina

(ASTO), 1024 IU; pH, 7.42; bicarbonate, 20 mEq/l; albumin, 28 g/l; cholesterolemia 200 mgr/dl; direct Coombs test, negative. A complete urine analysis revealed: nephrotic range proteinuria, 55 mg/m$^2$/h hemoglobin ++; hematic casts; thin and coarse granular casts and waxy casts. The results of a thorax examination were normal. Kidney ultrasound showed kidneys that were bilaterally enlarged with increased echogenicity. An enlarged liver and spleen was visible on the abdominal sonogram.

Forty eight hours after admission, the patient developed anuria; the creatinine increased to 5.2 mg/dl, and uremia increased to 210 mg/dl. Blood chemistry showed: Na, 137 mEq/l; K, 5.2 mEq/l; bicarbonate, 17 mEq/l; pH, 7.38. The exanthema was remitting. Intermittent peritoneal dialysis (IPD) was started, and three methylprednisolone pulses were administered at 10 mg/kg/dose due to the accelerated progression of his renal failure. Serum complement was: C3: 15 mg/dl (normal: 80–120), C4: 29.5 mg/dl (normal: 20–80); antinuclear antibodies, ANCA and rheumatoid factor were negative; a low titre for cryoagglutinins.

Serologic tests performed on the second day of hospitalization were negative for human immunodeficiency virus (HIV), toxoplasmosis, VDRL, hepatitis B, rubella, Epstein-Barr virus and parvovirus.

The detection of IgM antibodies against *M. pneumoniae* was positive.

The diuresis progressively returned after a few days, with improvement of renal function. IPD was withdrawn 5 days after admission. During hospitalization, a renal puncture biopsy was performed. The microscopic examination showed 13 glomeruli with increased mesangial cells and matrix, crowding of the polymorphonuclear leucocytes (PMNs) and double-outlined capillary basement membrane and a vascular area without changes. Immunofluorescence was positive for IgG, C3 and traces of IgM and IgA. The diagnosis suggested type I membranoproliferative glomerulonephritis. No electronic microscopy examination was performed (Fig. 1).

The identification by nested-PCR of nucleic acids in the extract obtained from the renal biopsy sample was positive for *M. pneumoniae* and negative for Parvovirus B19.

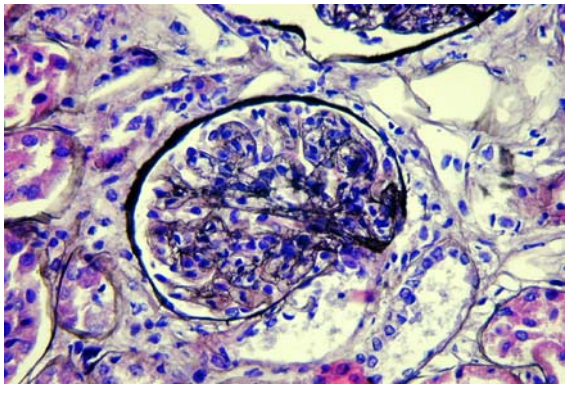

**Fig. 1** Renal biopsy (light microscopy): an increase in the mesangial matrix and cells, polymorphonuclear leucocytes (PMN) and capillary basement membranes reduplication are seen (Jones silver stains: 400×)

The patient was discharged 13 days after the admission in good general status, with normal renal function. Two months later, C3 and C4 levels had recovered and were 139 mg/dl and 37 mg/dl, respectively. After 1 year, his renal function is normal, without proteinuria or hematuria.

## Methods

The diagnosis of *M. pneumoniae* was based on an analysis of a serum sample obtained in the acute stage and on an analysis of a renal biopsy specimen.

*M. pneumoniae* was identified through the detection of IgM-class antibodies in the serum sample by means of immunofluorescence (Bion imprints, Bion Enterprises, Des Plaines, Ill.; Bion IgG, precipitating reactant, Bion Enterprises; Sigma fluorescein-labeled anti-human IgM, Sigma Chemical, St. Louis, Mo.) and confirmed through the detection of DNA in the raw extract of renal biopsy by nested-PCR. The biopsy sample was treated with a lysis solution (Tris, pH 8.3, proteinase K, Igepal and Tween 20). The DNA extracted from the biopsy sample was not purified, and the raw extract was used in the nested-PCR to amplify a segment of the genome that codifies for *Mycoplasma pneumoniae* cytoadhesion P1.

The raw extract was prepared to a final volume of 50 µl containing 10× PCR buffer, 2.0 m*M* $MgCl_2$, 2.0 U of *Taq* DNA polymerase (Invitrogen-Life Technology, Brazil), 2.0 m*M* dNTPs (Promega, Madison, Wis.), 0.02 µ*M* of each primer for the first round PCR (P1-40 and P1-331) and 0.2 µ*M* of each primer for the second round PCR (P1-178 and P1-285) and 10 µl of the extracted DNA.

The cycling conditions used in the first PCR round consisted of one denaturing cycle at 94°C for 1 min, 35 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s and lengthening at 72°C for 30 sec, with and one final lengthening cycle at 72°C. In the second PCR round, the number of cycles was increased from 35 to 40.

In the amplification reactions, a strain of *M. pneumoniae* Malbrán 002 was used as a positive control. As a negative control, one tube containing a reactant mixture with 10 µl of ultra-purified water as a template was used.

In order to develop the reaction products, 10 µl of the amplification products was loaded onto a 2.5% agarose gel with ethidium bromide. The gel was observed under ultraviolet light in order to detect the amplified products at 285 bp and 107 bp, which correspond to the first and second rounds, respectively (Fig. 2).

Analytical sensitivity of this technique was estimated in one copy of the genome using reference DNA (*M. pneumoniae* ATCC 15531 D).

## Discussion

Our patient presented with morbiliform exanthema and severe acute renal compromise, giving rise to the opinion that there were other etiologic factors in addition to the streptococcal infection. Though multiform erythema is the

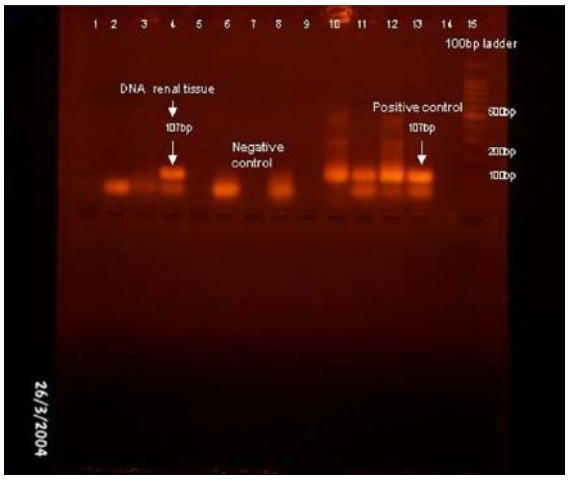


**Fig. 2** Ethidium bromide-stained 2.5% agarose gel showing the second round of *Mycoplasma pneumoniae* nested-PCR. *Lanes*: *2–4* Amplified non-respiratory samples, *6* negative control first and second round, *8* negative control first round, *10–12* respiratory samples, *13* positive control of 107 bp (first and second round). *Lane 15* 100-bp ladder

most common clinical type of exanthema associated with *M. pneumoniae*, other exanthemas have been associated to this infection as well [8].

We searched for the presence of Parvovirus B19 and *M. pneumoniae* based on such exanthema. The serum IgM test and kidney PCR were negative for Parvovirus B19 and positive for *M. pneumoniae*.

During the last 30 years, several reports of renal disease associated with *Mycoplasma* infections in children and adults have been published worldwide [9]. Eighteen cases have been reported in children [9, 10]. The *M. pneumoniae* antigen was detected by immunofluorescence in only two of all the patients reported in the literature [5, 6], and in other two cases the presence of the *Mycoplasma* antigen was demonstrated by immunoperoxidase staining of renal tissue [11, 12]. The sensitivity and specificity of PCR for the detection of tissular antigens are higher than those of other methods [13].

Said et al. [9] searched for *M. pneumoniae* antigens in renal tissue in three patients but obtained only negative results. However, they performed simple PCR; we believe that their negative results were due to the detection level of simple PCR generally being lower than that of nested-PCR [7].

The histological pattern seen in the renal biopsy – type 1 membranoproliferative glomerulonephritis – is the most frequently described one in the literature [9]. We considered that the diagnosis of post-streptococcal glomerulonephritis was improbable in our patient despite his increased level of ASTO. We did not have evidence of an ongoing streptococcal infection, or one that had occurred during the previous 7–10 days, throat swabs were negative and the patient did not have pyodermitis. Likewise, ASTO in children may increase up to 60% above normal levels without infection [14]. Anti-zymogen antibodies assay might have been useful, as these are more sensitive than ASTO in post-streptococcal glomerulonephritis [15]. On the other hand, the clinical evidence in association with renal compromise was not consistent with a post-streptococcal glomerulonephritis, for which the latency period is 7–10 days after the infection.

The determination of the mycoplasma antigen in the renal tissue together with the histology diagnosis were consistent with a membranoproliferative glomerulonephritis. Together with the IgG- and C3-positive immunofluorescence we were then able to suggest an immuno-pathogenic mechanism with immune complexes as the cause of glomerulonephritis in this patient.

## Conclusion

This case underscores the importance of a serologic and tissue search of the etiologic agent. To our knowledge, the case reported herein is the first one in which *M. pneumoniae* DNA has been demonstrated in a renal biopsy by means of a PCR technique in a child with positive serology and acute glomerulonephritis.

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