Case Report

Anti-Nuclear Antibody-Negative Lupus Nephritis or Post-Infectious Glomerulonephritis: Diagnostic Dilemma in a Young Male

Joyita Bharati, Saif Quaiser, Ritambhra Nada¹, Raja Ramachandran, Harbir Singh Kohli, Manish Rathi

Departments of Nephrology and Histopathology, Post Graduate Institute of Medical Education and Research, Chandigarh, India

Abstract
Proliferative lupus nephritis (LN) is histologically characterized by endocapillary hypercellularity and large immune deposits on light microscopy. Immunofluorescence shows almost all immunoglobulins and complement staining. The presence of antinuclear antibodies (ANA) is important for diagnosing systemic lupus erythematosus (SLE). Absence of ANA rules out the possibility of SLE according to the 2019 European League Against Rheumatism/American College of Rheumatology classification criteria for SLE. We report a young boy with fever, nephrotic-nephritic syndrome and pancytopenia consistent with hemophagocytic lymphohistiocytosis. Renal biopsy was consistent with LN; however, his initial ANA was negative. In view of pathological features of LN and persistent pancytopenia, high dose steroid therapy was started. Repeat ANA, done during the illness, turned positive. In this report, we describe the relevance of pathological patterns and the uncertainties of ANA positivity in making a diagnosis of SLE.

Keywords: Antinuclear antibodies negative, hemophagocytic lymphohistiocytosis, hyaline thrombi, lupus nephritis, systemic lupus erythematosus, wireloop lesion

Introduction
Proliferative lupus nephritis (LN) is characterized histologically by endocapillary hypercellularity and often large immune deposits on light microscopy.[1] Presence of antinuclear antibody (ANA) positivity is obligatory for classifying a patient as systemic lupus erythematosus (SLE) according to the 2019 European League Against Rheumatism (EULAR)/American College of Rheumatology (ACR) classification criteria.[2] We report a young boy who presented with fever, nephrotic-nephritic syndrome and pancytopenia. His renal biopsy was consistent with LN. However, ANA was initially negative leading to dilemmas surrounding the diagnosis of SLE in this case.

Case Report
A 15-year-old male presented to the clinic with complaints of fever for 3 weeks, swelling of the body for 2 weeks and reduced urine output for 1 week. Fever was intermittent up to 102°F. Swelling was noted around the eyes initially with gradual progression to involve the whole body with associated oliguria. He denied history of hematuria. There was no history of joint pain, oral ulcer, photosensitivity, malar rash, or alopecia. On examination, patient’s general condition was fair. His blood pressure was 138/90 mm Hg, pulse rate 88 per minute, respiratory rate 16 per minute and temperature was 100.2°F. General examination revealed pallor, right cervical and axillary lymphadenopathy and pedal edema. Lymph nodes were small (2 × 2 cm), firm, mobile, and non-tender. Per abdomen examination was noteworthy for hepatosplenomegaly (liver span of 16 cm and palpable spleen 2 cm below the costal margin) in addition to free fluid. Chest, cardiovascular and neurological systems were within normal limits. Laboratory parameters were as following: hemoglobin 7.3 g/dl, total leucocyte count 2800 per mm³, platelet count 64000 per µL, serum creatinine 1.7 mg/dl (150.28 µmol/L), serum albumin 1.2 g/dl (normal range: 3.4–4.6 g/dl) and 24-hour urine protein 6.5 g/day. Serum complement 3 (C3) and C4 were both low; while ANA, performed by indirect immunofluorescence assay (IFA) and enzyme-linked immunosorbent assay (ELISA), was negative. Repeat ANA, done during the illness, turned positive. Renal biopsy was consistent with LN; however, his initial ANA was negative. In view of pathological features of LN and persistent pancytopenia, high dose steroid therapy was started. Repeat ANA, done during the illness, turned positive. In this report, we describe the relevance of pathological patterns and the uncertainties of ANA positivity in making a diagnosis of SLE.

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negative. Hemolytic work up was negative except positive anti-IgG direct Coombs’ test. Tropical fever work-up, which included malaria, scrub typhus, leptospirosis, and Leishmaniasis, was negative. Blood and urine culture were sterile. Epstein Barr Virus serology and cytomegalovirus polymerase chain reaction were negative. Fever had resolved after 2 days of admission, however, pancytopenia persisted. Bone marrow aspiration and biopsy, done in view of persistent pancytopenia, revealed hemophagocytosis with no evidence of atypical cells. Cervical lymph node biopsy was suggestive of reactive lymphoid hyperplasia. In view of persistent nephrotic state with renal dysfunction and pancytopenia, possibilities of post-infectious glomerulonephritis and autoimmune disease (like SLE) were considered. After stabilization with packed cells and platelet transfusion, a renal biopsy was performed. The biopsy was suggestive of proliferative glomerulonephritis, with 5 out of 18 glomeruli showing cellular crescents, 12 glomeruli showing endocapillary proliferation, 14 glomeruli showing wire loop lesions and hyaline thrombi and interstitial fibrosis/tubular atrophy involving 10–15% of cortical area. Direct immunofluorescence showed coarse granular deposits of IgG (3+), IgA (2+), kappa (3+), lambda (3+), C3 (2+) and C1q (2+) along the capillary wall and mesangium. Electron microscopy showed subendothelial and mesangial deposits. Renal pathology was consistent with class IV LN. However, both ANA as well as anti-double stranded deoxyribonucleic acid (anti-dsDNA) were negative. In view of severe nephrotic state, oral prednisolone at a dose of 1 mg/kg/day was started. After 4 weeks of starting therapy, hematological abnormalities improved, serum albumin was 1.8 g/dl, serum creatinine was 1.4 mg/dl and 24-hour proteinuria declined to 4.5 g/day. Repeat serology and complements at this time revealed positive ANA (+2 homogenous, 1:80 dilution by IFA) and anti-dsDNA (42 IU/ml by ELISA) along with persistent low C3 and normal C4 levels. Low fixed dose cyclophosphamide as per Euro Lupus Regimen was started as an adjunctive immunosuppressive therapy as a diagnosis of SLE with LN (class IV) was now made. At the end of 3 months of therapy, patient was in partial remission with proteinuria of 1.8 g/day, serum albumin of 3.7 g/dl and serum creatinine of 0.8 mg/dl. His complete blood count showed hemoglobin of 11.8 g/dl, total leucocyte count of 5600 per mm$^3$ and platelet count of 2,18,000 per µL. Mycophenolate mofetil (1 g/day) along with oral steroids (5 mg/day) was started as maintenance therapy. At the last visit (5 months into illness), his serum albumin was 3.8 g/dl, serum creatinine 0.9 mg/dl and proteinuria was 0.89 g/day.

**Discussion**

We report a young boy with renal biopsy suggestive of LN and hematological manifestation of likely secondary hemophagocytic lymphohistiocytosis (HLH). We discuss the oddities which led to dilemmas in diagnosing SLE in this patient initially.

Presence of large immune deposits (wireloop lesions and hyaline thrombi) is characteristic of LN and/or monoclonal gammopathy of renal significance such as cryoglobulinemia. Recently, large IgA-dominant deposits (mostly in form of wireloop lesions) were described in patients with liver disease. LN was highly likely in our patient based on renal biopsy. However, the absence of ANA and anti-dsDNA positivity along with the lack of other musculoskeletal or mucocutaneous manifestations of SLE brought in uncertainties.

**Figure 1:** Photomicrograph shows global crescent (arrow) with membrano-proliferative pattern having wire loop lesion (arrowhead)

**Figure 2:** Direct immunofluorescence showing IgG (inset a), IgA (inset b), C3 (inset c), and C1q (inset d) staining
While the position of ANA positivity in diagnosing SLE has become centered in the 2019 EULAR/ACR classification criteria for SLE, the pitfalls of ANA detection using a particular kit, either IFA (Human Epithelial type-2 cells) or ELISA are also being realized. The variation in ANA detection using different assays in established SLE patients and a significant proportion of ANA negative SLE patients included in clinical trials are supportive evidence in this regard. ANA positivity is likely dependent on the type of assay and kit used for detection, stage of the disease and/or age of the affected population. In the published literature on ANA-negative LN, 40% were children in whom a different mechanism of autoimmunity leading to SLE is being postulated. In those who initially had negative ANA serology and later developed ANA positivity, a gap of few months to 10 years was observed. The cause for such a phenomenon is not clear. Our patient developed ANA and anti-dsDNA positivity in low titers after around 8 weeks of illness and 4 weeks of starting therapy. Patient fulfilled 3 clinical (renal, leucopenia, thrombocytopenia) and 4 immunological (positive ANA, positive anti-dsDNA, low complements and positive direct Coombs test) Systemic Lupus International Collaborating Clinics Classification (SLICC) Criteria for SLE in addition to the renal biopsy findings suggestive of LN. The 2019 EULAR/ACR criteria were also subsequently fulfilled with a score of 29. Thus, he was started on intravenous cyclophosphamide as an adjunct to steroid therapy to which he responded with partial remission.

The pitfalls of diagnosing LN based on histopathology patterns was highlighted recently by Kudose et al. where they described the rare possibility of non-lupus glomerulonephritis even when specific LN criteria are met. “Full house” staining, though characteristic of LN, is not universally present. So, only biopsy findings of LN are inherently flawed in diagnosing SLE in the absence of immunological or clinical parameters.

Our patient had aggressive pathology patterns such as crescents, diffuse wireloop lesions and hyaline thrombi with immunofluorescence showing intense IgG, C3, and C1q staining making the possibility of LN very likely. Subsequent ANA positivity in follow-up made the diagnosis clear. The importance of prompt diagnosis and start of therapy in patients with LN is explicit. The oddities of SLE in our patient which led to diagnostic dilemmas were (1) occurrence in a 15-year-old young boy, (2) lack of common extra-renal manifestations of SLE, which occurs more in those with LN than those without, and (3) negative ANA during initial presentation. While ANA-negative LN or lupus-like nephritis is reported previously, 2019 EULAR/ACR classification criteria has dismissed it.

**Conclusion**

This report highlights the clinical implications of renal biopsy findings and puts forward a case for reconsidering the position of ANA positivity in diagnosing SLE in a given patient. It is possible that the current understanding of ANA positivity in SLE patients is incomplete. Future research on validation of ANA assays and kits across population with diverse ethnicity/race and at different stages of the disease may clarify the reason for variation.

**Patient consent**

A written informed consent was taken from the patient.

**Declaration of patient consent**

The authors certify that they have obtained all appropriate patient consent forms. In the form the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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**Conflicts of interest**

There are no conflicts of interest.

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