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IM - CASE RECORD

A 79 year old man with chronic lymphocytic leukemia and nephrotic syndrome

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Case presentation

Dr. Netti, Dr. Bruno A 79-year-old man was diagnosed with B-cell chronic lymphocytic leukemia (B-CLL) in another institution. During hospitalization, hematochemical examination revealed leukocytosis (30.8 × 10^9 cells/L) with inverted formula (neutrophils 26%, lymphocytes 64%) and chronic renal failure [serum creatinine 143.2 μmol/L; estimated glomerular filtration rate (eGFR), 54 mL/min/1.73 m^2 according to the modification of diet in renal disease (MDRD) study equation], while hemoglobin level and platelet count were normal. At physical examination, no painful disease-related overgrowth of lymph nodes or spleen was observed. Only a few percentage of peripheral blood lymphocytes showed an undifferentiated phenotype (<10%). Because of the low grade disease (B-CLL RAI 0, Binet A), no treatment was carried out.

Six months later, he developed pre-tibial oedema and asthenia. Blood analyses showed an increase in serum creatinine level to 230.7 μmol/L (eGFR, 31 mL/min/1.73 m^2), and urinalysis showed 100–150 red blood cells/high-power field (hpf) and severe proteinuria (6.96 g/day). At this point, the patient was admitted to our hospital. Hematochemical examination showed leukocytosis (24 × 10^9 cells/L) with an inverted differential count (neutrophils 8.8%, lymphocytes 80.2%), while hemoglobin level, hematocrit, and platelet count were normal. International normalized ratio of prothrombin was 1.07; activated partial thromboplastin time was 36.1 s; and fibrinogen level was 375 mg/dL (11.025 μmol/L). Total protein level was 54 g/L, albumin level was 23 g/L, α-2-globulin level was increased, and γ-globulin level was decreased. Serum levels of IgG and IgM were reduced (395 mg/dL and 8 mg/, respectively); IgA and complement levels were normal. Sedimentation rate was 4 mm/h with a C-reactive protein level of 3.39 mg/dL. Serum β-2-microglobulin was increased (0.1063 g/L). Serum creatinine level was 229.8 μmol/L (eGFR, 31 mL/min/1.73 m^2), and blood urea nitrogen level was 21.42 mmol/L. A 24-h urine collection had 4.16 g of proteins. Urine sediment contained many red blood cells (100–150/hpf) and leukocytes (50–100/hpf) with granular casts. Therefore, nephrotic syndrome was diagnosed.

To stage the hematological disease, a peripheral blood cell morphology was examined, which confirmed an inverted differential leukocyte count (neutrophils 15.0%, lymphocytes 84.1%) and showed many Gumprecht ghosts. The flow-cytometric analysis of peripheral blood lymphocyte subsets revealed that the leukemic cells were positive for CD19 (88%), CD20 (83%), and CD23 (67%).

Dr. Di Palma A kidney biopsy was performed. Light microscopy sections showed 11 glomeruli, four of which being fully sclerotic. Marked endocapillary proliferation with increased mesangial cellularity and matrix, and lobular simplification were present (Fig. 1a). Moreover, subendothelial deposits, resulting in a thickened capillary wall

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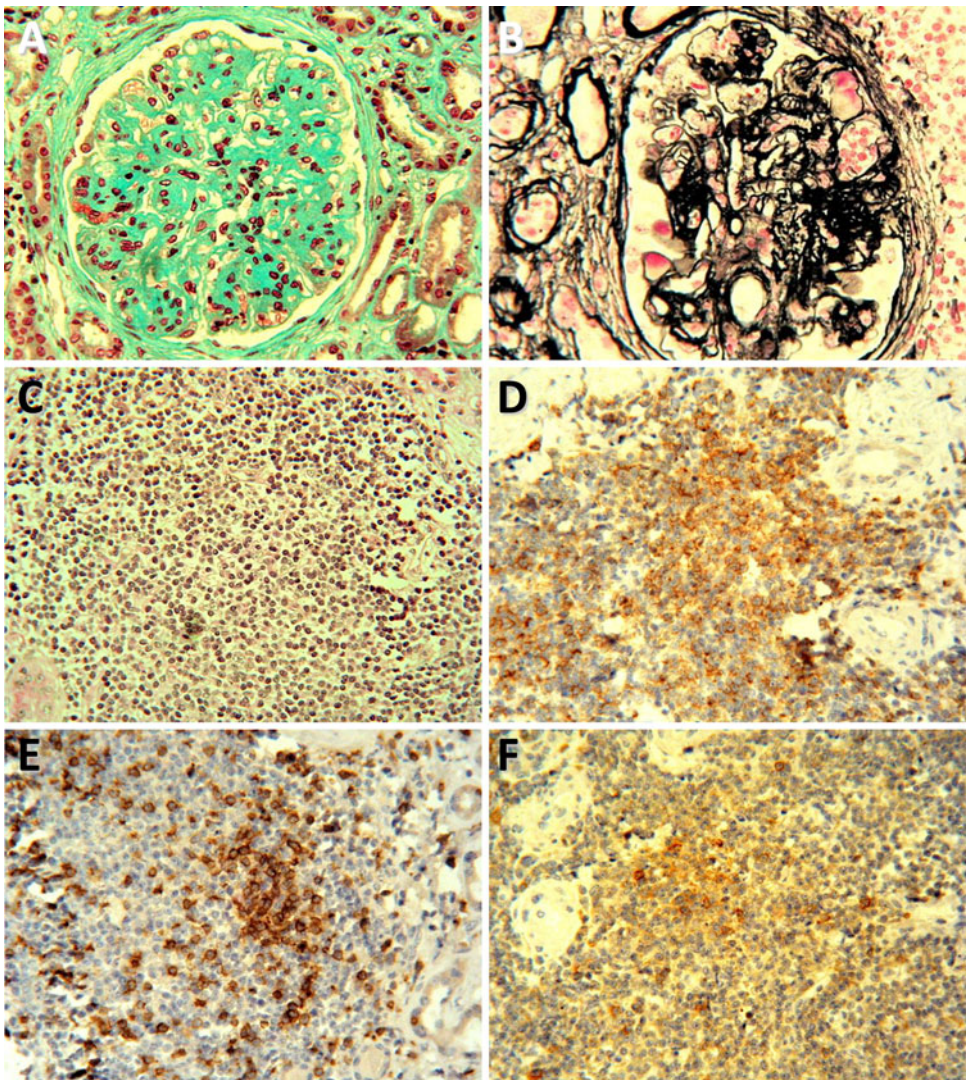

Fig. 1 Light microscopy of renal biopsy specimen sections: **a** Light microscopy image showing typical membranoproliferative glomerulonephritis (MPGN) characterized by diffuse endocapillary proliferation with increased mesangial cellularity and matrix, and lobular simplification (Trichrome stain; ×400). **b** Extensive double contours of the glomerular basement membranes, stained by silver, caused by mesangial interposition and new basement membrane formation in response to subendothelial immune complex deposits were visible. The deposits were PAS positive and occasionally appeared as pseudothrombi. There was also a focal segmentary adhesion (synechiae) of the capillary tuft to the Bowman's space (Jones' silver stain, ×400). **c** Tubular interstitial examination revealed large interstitial infiltrates of homogeneous small mononuclear cells (MNCs) with hyperchromatic nuclei (Hematoxylin–Eosin, ×400). Immunohistochemistry on renal biopsy specimen showing that infiltrating MNCs were evenly CD20 + (**d** *red*) while some cell areas at the infiltrate periphery were CD3 + (**e** *red*) and seldom CD5 + (**f** *red*) (Original magnification: ×400)

and a double contour of the glomerular basement membrane with occasional appearance of pseudothrombi, and focal segmentary adhesions (synechiae) of the capillary tuft to Bowman's space were observed (Fig. 1b). There were no crescents. A severe interstitial fibrosis was present, with diffuse flattening of the epithelium, while blood vessels showed intimal fibrosis and mild hyalinosis. The immunofluorescence findings were diffuse parietal deposits of C3 and IgG with predominance of staining for kappa over lambda light chains Tubular interstitial examination revealed diffuse tubular atrophy and interstitial fibrosis. Thus, a diagnosis of membranoproliferative glomerulonephritis (MPGN) type 1 was made.

In addition, large interstitial infiltrates of homogeneous small mononuclear cells (MNCs) with hyperchromatic nuclei were observed (Fig. 1c).These histological findings suggested a massive renal involvement by leukemic cells. Thus, immunohistochemistry was performed on cryostat sections obtained from the renal biopsy specimen, which showed that infiltrating MNCs were prevalently CD20+ (B lymphocytes), while some cell areas at the infiltrate periphery were CD3+ (T lymphocytes) and seldom CD5+,

suggesting that these cells might belong to the original leukemic population (Fig. 1d–f), despite the presence of inflammatory T-cells.

Further investigations

Dr. Gigante, Dr. Prattichizzo To provide direct evidence of MNCs infiltrate clonality, frozen renal biopsy samples were subjected to laser capture microdissection (LCMD) [4]. Sections of identical dimensions were obtained from infiltrating MNCs and kidney tissue areas of the same biopsy specimen, in which MNCs were absent (Fig. 2a–e). DNA from LCMD tissue fractions was analyzed by qualitative polymerase chain reaction (PCR)-based detection of clonal gene rearrangements of the immunoglobulin heavy chain gene (IGH), using IdenticloneIgH Gene Clonality Assay [5]. Peripheral blood leukemic CD19+ CD20+ lymphocytes obtained by magnetic immunolabeled sorting were also used as positive control.

GeneMapper analysis of polyclonal DNA control, performed using IGH Tube A Master Mix targeting the Framework region of IGH gene, showed a Gaussian distribution of multiple peaks, representing many different PCR products within an expected size range (310–360 bp) and reflecting the heterogeneous population of rearrangements (Fig. 2f). Monoclonal DNA control showed instead a single peak, as expected (Fig. 2g). PCR analysis of DNA from kidney biopsy specimen of the patient, in which no infiltrating MNCs were detectable (blue selected area, Fig. 2b, d), showed no peak. This demonstrated that no lymphocyte DNA was present (Fig. 2h). Unlike normal kidney, renal tissue with infiltrating cells (red selected area, Fig. 2b, e) showed a monoclonal single peak of 343 bp (Fig. 2i), which was also detectable in peripheral blood leukemic CD19+ CD20+ lymphocytes of the patient (Fig. 2j), thus definitely demonstrating the leukemic (clonal) origin of renal interstitial MNC infiltrate in our patient. The same results and a perfect reproducibility were obtained for all other Master Mixes (IGH Tube B-D) of IdenticloneIgH Gene Clonality Assay, which showed monoclonal peaks of 278, 142, and 140 bp; data not shown).

Clinical diagnosis

Dr. Netti MPGN type 1 associated with massive tubular interstitial infiltrate secondary to B-cell Chronic Lymphocytic Leukemia.

Discussion

Dr. Infante, Dr. Stallone Chronic Lymphocytic Leukemia (CLL) is a chronic lymphoproliferative disorder characterized by a progressive accumulation of morphologically mature but functionally incompetent lymphocytes, which are monoclonal in origin. CLL is manifested by progressive accumulation of these cells in the blood, bone marrow, and lymphatic tissues [1]. Lymphocyte counts are usually $\geq$5,000/mm^3 with a characteristic immunophenotype (CD5 and CD23 positive B-cells) [1].

The appearance of an isolated mononuclear cell (MNC) infiltrate in the kidney is a common occurrence in this disease, however, it is an extremely rare cause of nephrotic syndrome and acute renal failure. The majority of B-CLL patients with nephrotic syndrome show secondary MPGN type 1, characterized by discrete immune deposits in the glomerular capillary wall [2]. Other histologic lesions associated with nephrotic syndrome during CLL may be membranous glomerulonephritis, minimal change, crescentic glomerulonephritis, light-chain deposition disease, amyloidosis, and focal segmental glomerulosclerosis [2].

Several reports have described renal infiltration by lymphoid cells in B-CLL, which may be immunophenotypically characterized as leukemic infiltrates, but until now monoclonal origin of infiltrating MNC has not been described.

Noteworthy, during B-cell ontogenesis, the B-cell receptor (BCR) gene undergoes rearrangement in both heavy and light chains. These genes are clonally rearranged, and are found in the B-cell clone progeny, even after neoplastic transformation [3]. Thus, PCR assays by multiple consensus DNA primers targeting gene rearrangements of the immunoglobulin heavy chain gene (IGH) might clearly show the clonal origin of renal interstitial cell infiltrate.

In this report, we clearly demonstrate by a molecular approach, the monoclonal origin of massive MNC infiltrate of the kidney in a patient with B-CLL.

Conclusions

Prof. Gesualdo In summary, tubular interstitial infiltration of the kidneys is a common feature in many inflammatory and neoplastic diseases, but when other characteristic clinical signs are lacking, the isolated presence of a MNC infiltrate in the kidney represents a very challenging diagnosis based on currently available diagnostic assays. This study combines the classical histological approach with LCMD and PCR-based detection of clonal IGH gene rearrangements as a rapid and highly specific diagnostic tool to definitely show that kidney infiltrating MNCs in a patient with B-CLL are derived from the original leukemic population and to clearly exclude the possibility of a kidney inflammatory disease. This novel approach might be suitable for the diagnosis of different clinical conditions

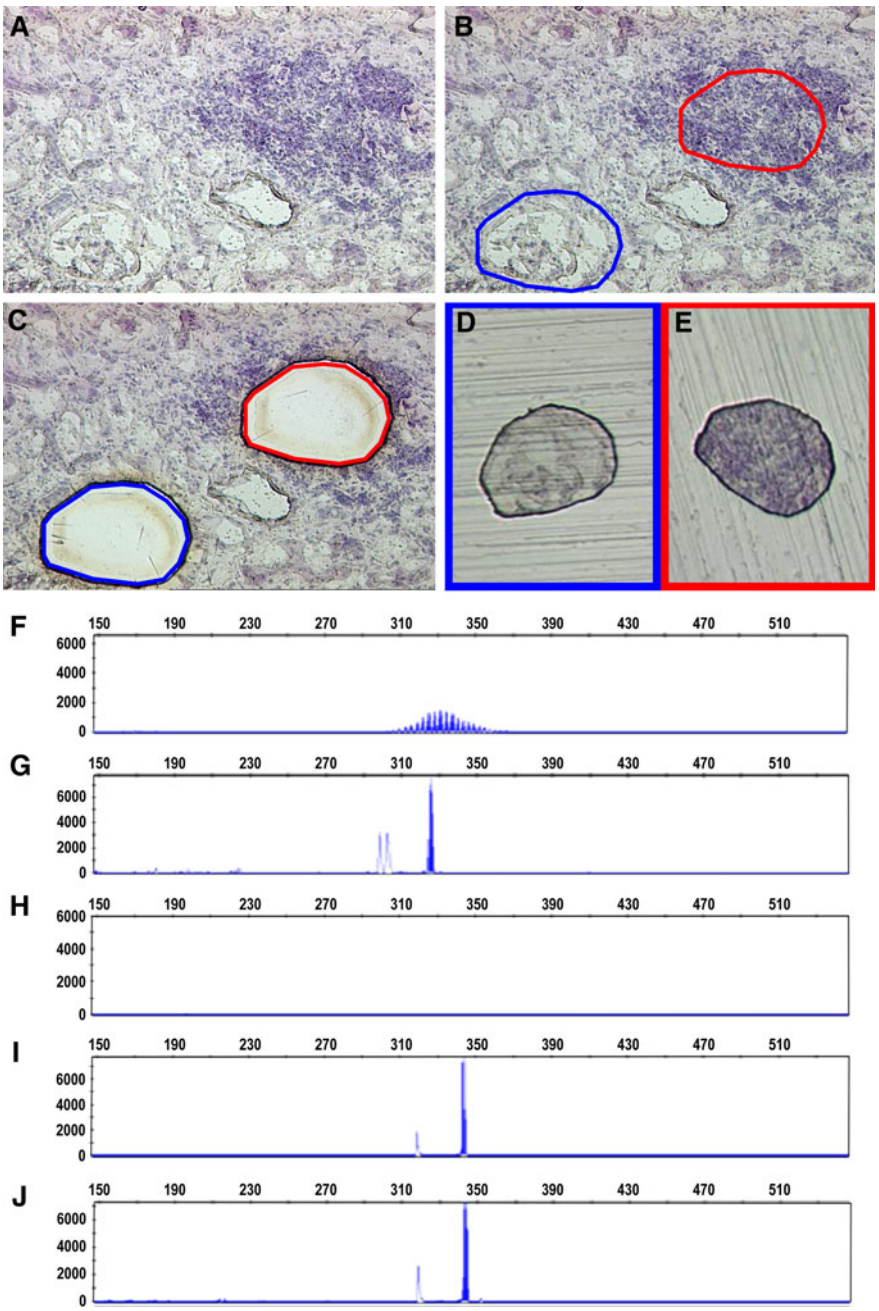

Fig. 2 Laser capture microdissection of MNC infiltrate combined with qualitative PCR-based detection of clonal gene rearrangements of the immunoglobulin heavy chain gene (IGH). **a** Frozen renal section from patient's kidney biopsy showing MNC infiltrate surrounded by normal renal tissue (Original magnification: ×200). Infiltrating MNCs (*red area*) were recovered from renal biopsy specimen by using a Leica Laser Microdissection System (LMD AS, Leica Microsystems, Wetzlar, Germany) on an area of 32.625 μm^2 (**b, c** *red area*) and an identical area was also recovered from a zone free of infiltrating MNCs (**b, c** *blue area*) (**b**, **c** ×200). In all the experiments, serial 4-μm-thick cut sections were mounted on a polyethylene naphthalate membrane slide (Leica Microsystems) and selection of cells for LCMD was guided by Hematoxylin and Eosin staining. All the experiments were repeated thrice. After LCMD procedure, normal and infiltrated tissue specimens (*blue* and *red selected area*, respectively) were harvested in separated caps containing the Qiagen (Hilden, Germany) lysis buffer for nucleic acid extraction (**d**, **e**: ×200). Data generated using IGH Tube A Master Mix containing multiple oligonucletides targeting the framework 1 region of the immunoglobulin heavy chain gene (IdenticloneIgH Gene Clonality Assay) showing a bell-shaped curve of amplicon products in the Polyclonal Control DNA (Valid Size Range 310–360 bp) (**f**) and a single peak in Monoclonal Control DNA (Valid Size 325 bp) (**g**). PCR analysis of DNA from patient showing no peak from renal tissue without infiltrating cells (**h**), a monoclonal single peak of 343 bp renal tissue with infiltrating cells (**i**), and the same monoclonal single peak of 343 bp from peripheral blood leukemic CD19 + CD20 + lymphocytes (**j**). Data generated using IGH Tube A Master Mix containing multiple oligonucletides targeting the framework 1 region of the immunoglobulin heavy chain gene (IdenticloneIgH Gene Clonality Assay)

characterized by infiltrating MNCs of the kidney, for a prognostic point of view and for the therapeutic approach.

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Conflicts of interest None.

References

1. Dighiero G, Hamblin TJ (2009) Chronic lymphocytic leukaemia. Lancet 371:1017–1029
2. Moulin B, Ronco PM, Mougenot B, Francois A, Fillastre JP, Mignon F (1992) Glomerulonephritis in chronic lymphocytic leukemia and related B-cell lymphomas. Kidney Int 42:127–135
3. Janeway CA, Travers PW, Walport M,Shlomchik M (2005) The rearrangement of antigen receptor gene segments controls lymphocyte development. In: Immunobiology, 6th edn. The immune system in health and disease. Garland Science, New York, pp 258–275
4. Lazzeri E, Netti GS, Mazzinghi B, Cirami C, Salvadori M, Romagnani P (2006) A young woman with oedema. Intern Emerg Med 1:209–215
5. van Dongen JJM, Langerak AW, Bruggemann M et al (2003) Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: Report of the BIOMED-2 Concerted Action BMH4-CT98–3936. Leukemia 17:2257–2317