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## UN CASO DI GLOMERULONEFRITE CRIOGLOBULINEMICA VS IMMUNOTATTOIDE

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Most renal diseases with organized deposits are relatively uncommon conditions. A right diagnosis requires a multi-disciplinary approach in which clinical history and pertinent laboratory data play a fundamental role. The renal biopsy is mandatory for a precise nosological classification. Light microscopy and immunofluorescence lead the diagnostic investigation but the ultrastructural examination could be crucial in resolving diagnostic dilemmas. In this relation a case of a 58 old-year male with a history of chronic renal failure and nephrotic-range proteinuria will be presented. The patient underwent three kidney biopsies within three years, each showing a glomerulopathy with organized deposits, morphologically suggestive for cryoglobulinemic nephropathy. No cryoglobulins were found in serum. Also the research of the autoantibodies and the markers of viral hepatitis were negative. The clinical history of the patient was unremarkable for neoplasms, immune disorders or infective pathologies. Considering these clinical data immunotactoid glomerulonephritis was hypothesized. This case represents a diagnostic challenge in definitions of glomerulopathies and highlights the possible overlap that concern these rare disorders.

## A CASE OF LENVATINIB-INDUCED RENAL FAILURE AND REVIEW OF THE LITERATURE

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Lenvatinib is an orally bioavailable multi-tyrosine kinase inhibitor (TKI) of VEGFR, FGFR, PDGFR- $\alpha$ , KIT and RET, and is the most effective drug for advanced progressive Iodine-131 refractory (RAI) differentiated thyroid cancer patients. Proteinuria and renal failure (RF) were reported among the most frequent LEN-induced adverse events (AEs), often leading to discontinuations or dose modifications. We described in this paper a case of LEN-induced renal failure with severe proteinuria in a man treated for a metastatic papillary thyroid carcinoma. Kidney biopsy showed a glomerular damage secondary to Lenvatinib therapy. Light microscopy examination revealed a huge number of glomeruli with some

degree of mesangial hypercellularity and increase of mesangial matrix. In just one glomerulus, segmental features of mesangiolysis were observed. Capillary basal membranes were thickened for the presence of double contours. Moreover, arteriolar narrowing due to intimal edema, endothelial swelling and focal sub endothelial necrosis was found. No inflammatory reaction was present in the vessels walls. The interstitial showed fibrosis and tubular atrophy. The tubulointerstitial nephropathy was also supposed by clinical evaluation and laboratory tests. Immunofluorescence analysis revealed weak focal and segmental staining for immunoglobulins and complement along the capillary basal membrane.

Ultrastructural examination showed focal sclerosis, focal podocyte foot process effacement and rare electron dense deposits with subendothelial and intramembranous localization. The diagnosis was of tubulointerstitial and vascular necrotic damage, associated with endothelial damage described as thrombotic microangiopathy-like pattern. Moreover, ultrastructural data suggest also the presence of podocyte injury, with foot process effacement.

Effective management was obtained by oral steroids without interrupting LEN.

All these aforementioned features are indicative of drug-derived damage; anti Vegf therapies can induce thrombotic microangiopathy and podocytopathies. In our patient we found a combination of both lesions, associated with an diffuse tubulointerstitial involvement.

## ATOMIC FORCE MICROSCOPY (AFM): AN ADDITIONAL VALUE TO ULTRASTRUCTURAL DIAGNOSTICS

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Ultrastructural studies with transmission electronic microscope (TEM) saw their hey-days in the '70 providing a relevant contribution not limited to pathophysiology but also in daily diagnostics. Often wrongly considered expensive and time-consuming, requiring a differentiated fixation, embedding and cut at the ultramicrotome, the usage of TEM is declining in pathology labs. TEM often remain pivotal to achieve a final correct diagnosis in kidney pathology. With this very preliminary report we would share the experience of our multidisciplinary study groups composed by physicians, biologists and bio-medical engineers proposing a new intriguing tissue imaging technique. With Atomic Force Microscopy we are able to obtain an ultrastructural imaging of renal tissue using routinely formalin fixed, paraffin embedded kidney biopsies cutting 3- $\mu$ m slides with a common rotative microtome. The atomic force microscopy (AFM) belongs to a series of scanning probe microscopes (SPM) invented in the 1980s. The functioning principle consists on measuring the interaction forces into a very sharp tip mounted to a cantilever spring in close proximity to the sample by monitoring the deflection of the cantilever. It is possible to obtain a topographic image of the sample by plotting the deflection of the cantilever versus its position on the sample during scanning.

AFM-based imaging evidences amazingly each single component of the renal corpuscle. Small elements as red cells inside of glomerular capillaries can be clearly identified, as well

as mesangium, juxtaglomerular apparatus, and podocytes. Furthermore, this technique allows to observe relationships between different components and plot topography profiles from particular selected zones, especially for measuring distances as thickness, diameters, or spaces.

Details about structure and morphology of small components can be evidenced in AFM imaging by zoom-in scanning. This procedure is possible by selecting the zoom-area for scanning from a previous bigger image, then the zoom-in inversely increases in respect of the scanning- area size. Variables as temperature, humidity, tip shape, scan-speed, etc., become determinants for the quality of the zoom-in image. Small structures' details of the kidney tissue were magnified by applying zoom-in scanning. Intra-glomerular structures as pedicels of podocytes, mesangial cell processes, red cells inside of capillaries; and extra-glomerular structures as Bowman's space, parietal layer of the Bowman's capsule, mesangium, and juxtaglomerular surface roughness, can be remarkably identified. It was also possible to perform real tridimensional (3D) views of the scanned AFM-images from Z-axis data.

The wide resolution range of AFM permits to evaluate samples from the micro-scale to the nano-scale. This characteristic is ideal for studying biological samples and identifying ultrastructural relationships existing between different components of tissues and cells, especially by 2D and 3D structural analyses.

The role of AFM in diagnostics will be elucidated in further studies but the proposed technique appear promising and may represent a new chapter in kidney pathology.

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## Venerdì 19 ottobre 2018

Sala Nico 2 – 08:00 - 10:00

### PATOLOGIA SPERIMENTALE

## Metabolismo ed Immunoregolazione nell'era dei Checkpoint Inhibitors

Moderatori: M. Ponzoni, C. Tripodo

### IMMUNOMETABOLIC CHECKPOINTS OF REGULATORY T CELL DYNAMICS IN CANCER

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Though representing a minor proportion of CD4 T lymphocytes in tissues in normal conditions, Tregs undergo dynamic processes of contraction or expansion in response to external signals, thus respectively unleashing or restraining immune effector cells, and critically impacting on protective as well as on immunopathological reactions.

Treg expansion is a key event in tumor immune escape, being Treg infiltration associated with a worse prognosis in most cancers. Much effort is being devoted to the identification of consensus signature of tumor-associated Tregs, in order to identify selective targets for their inactivation. Moreover, in the tumor microenvironment, Tregs are exposed to a variety of signals that may either stabilize or undermine their suppressive activity.

OX40 is a member of the tumor necrosis factor receptor superfamily, whose expression marks a human Treg subpopulation endowed with phenotypical, functional and epigenetic features of suppressive function, proliferation and stability, which is particularly expanded in human cancers. We have recently revealed that Tregs' advantage in the tumor milieu relies on supplemental energetic routes involving lipid metabolism, and that OX40-expressing tumor-infiltrating Tregs displayed a gene signature oriented toward glycolysis and lipid synthesis. Therefore, immunometabolic routes may contribute to shape Treg-mediated immune regulation in cancer, thus representing suitable targets to rescue immune surveillance.

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### METABOLISM AND IMMUNOSUPPRESSION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world and is characterized by accumulation of mature B cells in the peripheral blood and in the lymphoid organs. Disease outcome is influenced by both a complex pattern of genetic lesions and by a network of stimuli coming from non tumoral neighboring cells in the microenvironment. Tumor-host interactions are particularly