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RED BLOOD CELLS FOUND IN THE URINE: PROPOSED DIAGNOSTIC PROTOCOLS TO UNVEIL THEIR ORIGIN

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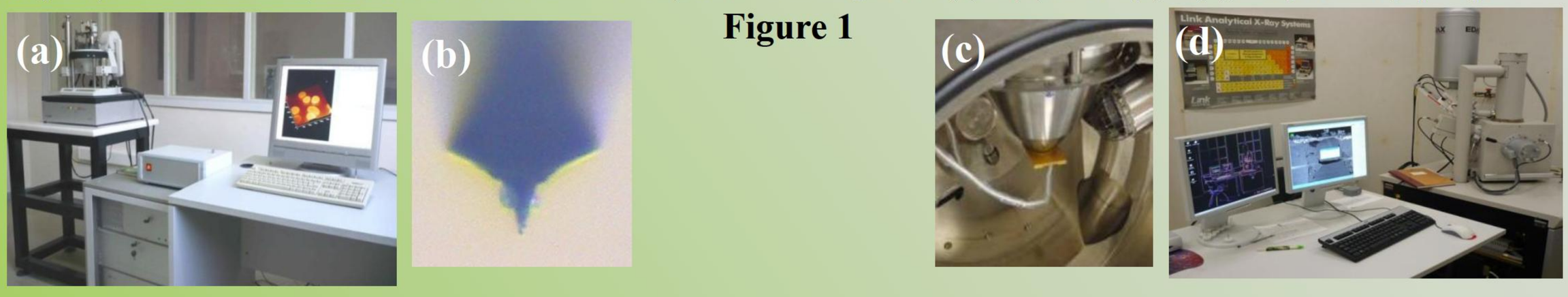
INTRODUCTION AND AIMS: The existence of red blood cells (RBC) in the urine (uRBC), hematuria, can originate from different underlying diseases of the upper (kidneys/ureters) or the lower (bladder/urethra) urinary system [1]. Today, there is no overall consensus regarding the reliability of the RBC morphology as a diagnostic tool to identify the area of the release. Here we propose relatively easy protocols that can employed in clinical practice for the investigation of the uRBC origin.

METHODS: uRBC obtained from 24h-collection/spot samples were investigated in comparison to peripheral blood RBC (pbRBC). Patients diagnosed with glomerulonephritis (GN) (N=2) were contrasted to healthy donors (N=2) to investigate the mechanisms that motivate/promote or reverse any dysmorphy that can be possibly observed. The following protocols were employed: (I) uRBC and pbRBC studied in intact form. (II) pbRBC matured in autologous urine (pbRBC-u) at T=25 ^{o}C up to 4 hours, to simulate an artificial bladder environment to investigate the action of purely biochemical/osmotic mechanisms (motivation/promotion of dysmorphy). (III) uRBC matured in autologous blood plasma (uRBC-bp) at T=25 ^{o}C up to 4 hours, to simulate an artificial cardiovascular environment to investigate the action of purely biochemical/osmotic mechanisms (reversal of dysmorphy). Smears obtained from these three protocols, prepared with only minimal processing, were imaged with the conventional optical microscope (OM; resolution level μm=10^{-6}m) and the advanced Atomic Force and Scanning Electron Microscopes (AFM and SEM; resolution level nm=10^{-9}m) shown in Figures 1(a)-1(b) and 1(c)-1(d), respectively [2-3].

Figure 1



RESULTS: The imaging with OM, AFM and SEM revealed that: (a) uRBC of the GN patients were not necessarily dysmorphic. (b) pbRBC-u of the GN patients exhibited intense coagulation with concurrent loss of cytoplasm (horizontal arrows in Figures 2(a)-2(b)), while for the healthy donors, pbRBC-u exhibited mild coagulation and limited spherocytosis, however, without loss of cytoplasm (Figures 3(a)-3(b)). (c) uRBC-bp showed no significant alterations in both GN patients and healthy donors.

Figure 2

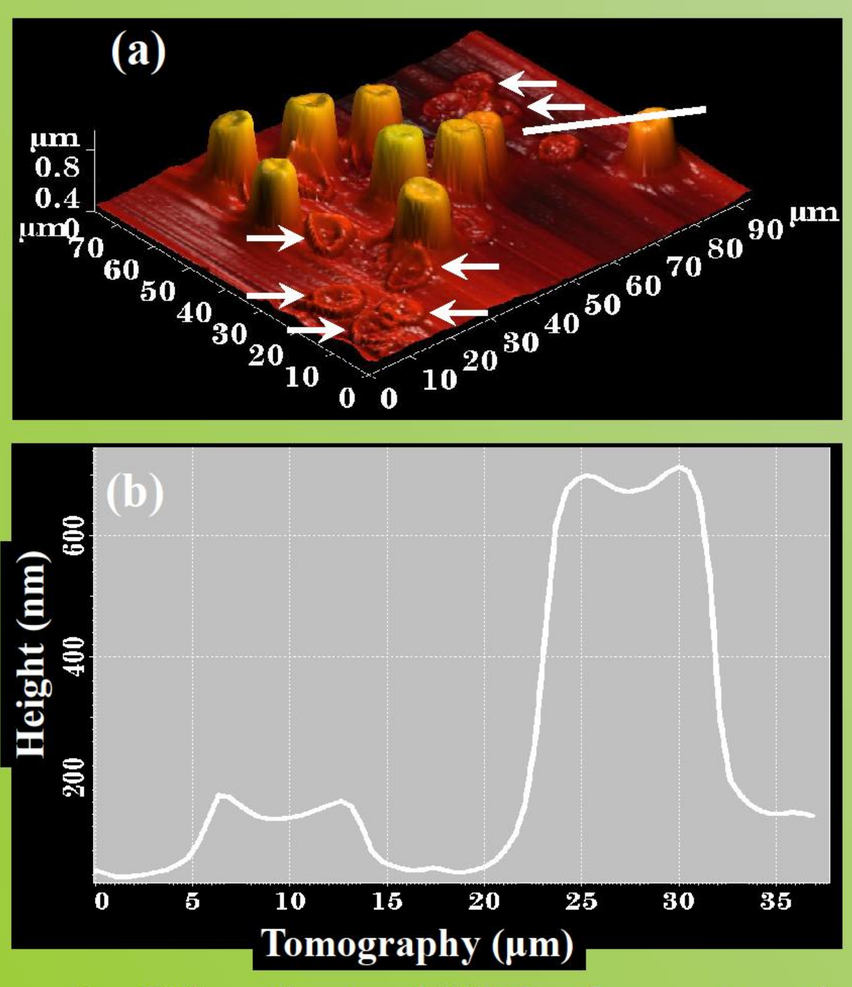


Figure 2. GN patient: pbRBC after maturation in autologous urine (pbRBC-u). (a) AFM image in three-dimensional rendering. Horizontal arrows indicate partially lysed pbRBC-u. (b) Tomography along the white line of panel (a).

Figure 3

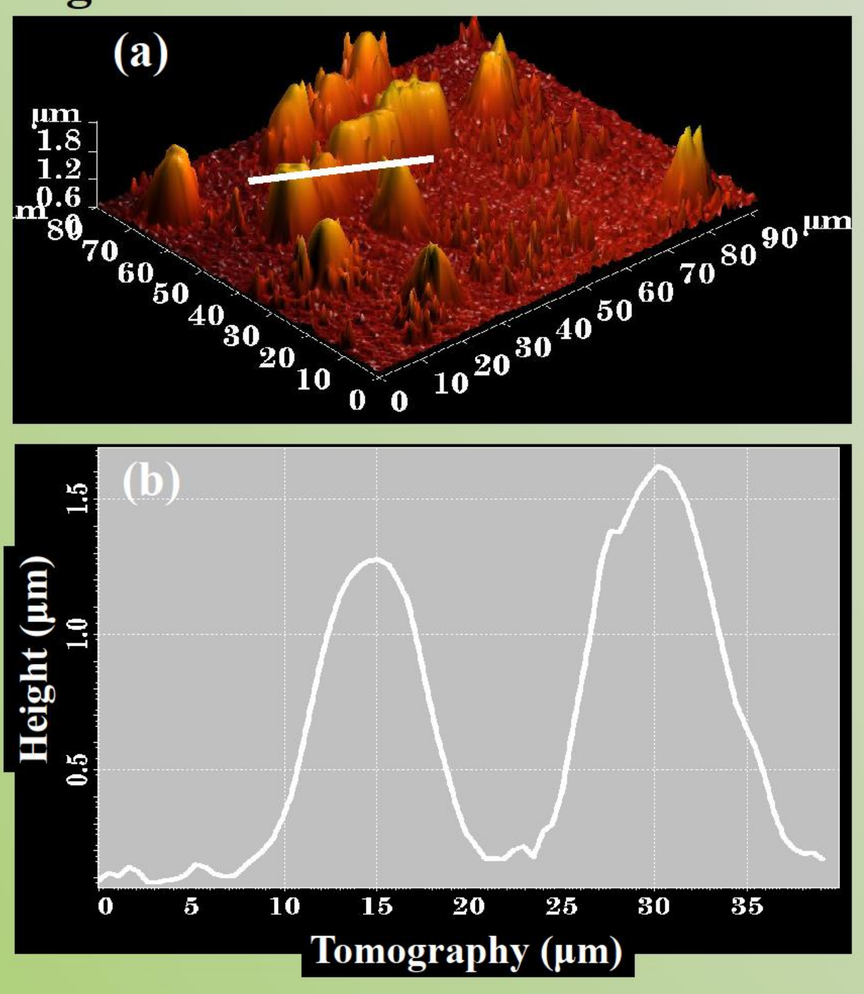


Figure 3. Healthy donor: pbRBC after maturation in autologous urine (pbRBC-u). (a) AFM image in three-dimensional rendering. All pbRBC-u exhibit spherocytosis. (b) Tomography along the white line of panel (a).

CONCLUSIONS: These introductory experiments, that are based on specially designed, however easy to perform, experimental protocols evidenced clear differences in the morphology of RBC between the GN patients and healthy donors. We believe that such protocols could be employed in clinical practice to investigate reliably the identity of hematuria.

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