51st ERA-EDTA CONGRESS AMSTERDAM THE NETHERLANDS MAY 31st JUNE 3rd 2014

ULCER-LIKE DECONSTRUCTIONS OF THE RED BLOOD CELL MEMBRANE INDUCED BY THE URAEMIC MILIEU IN HAEMODIALYSIS PATIENTS

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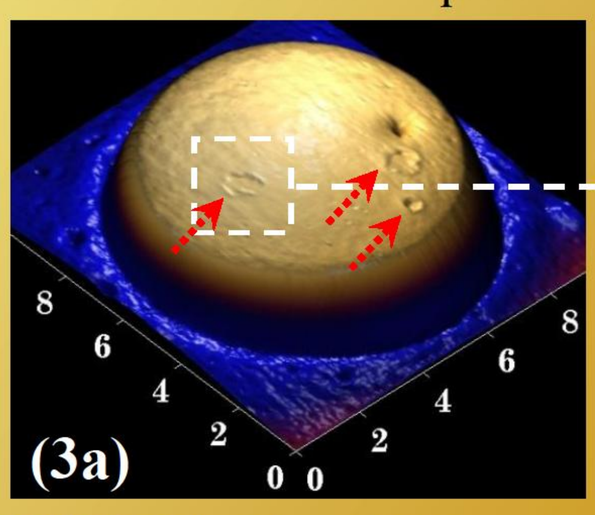
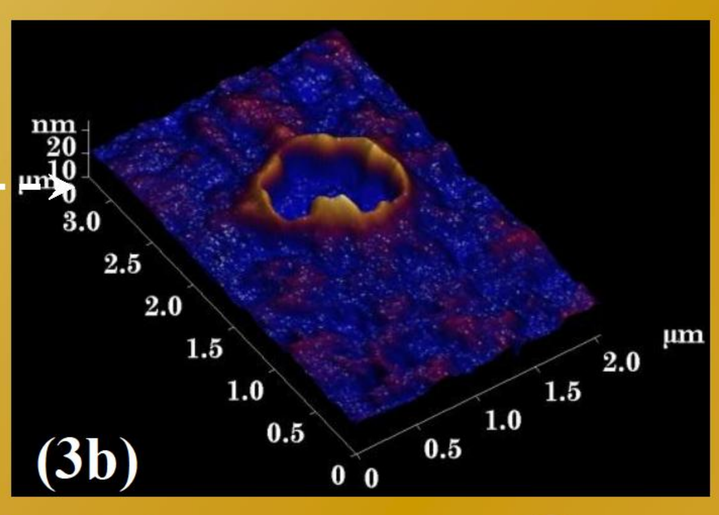
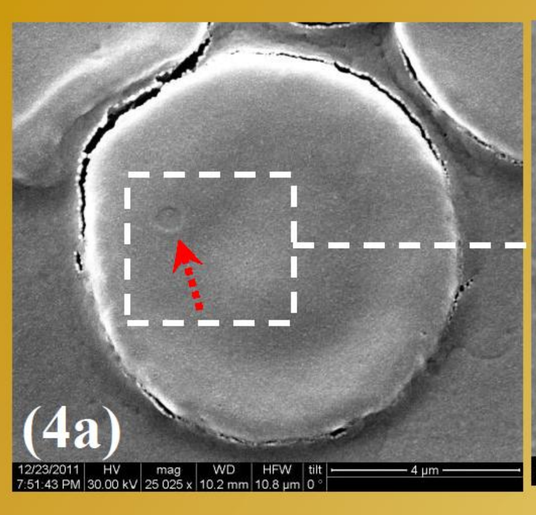
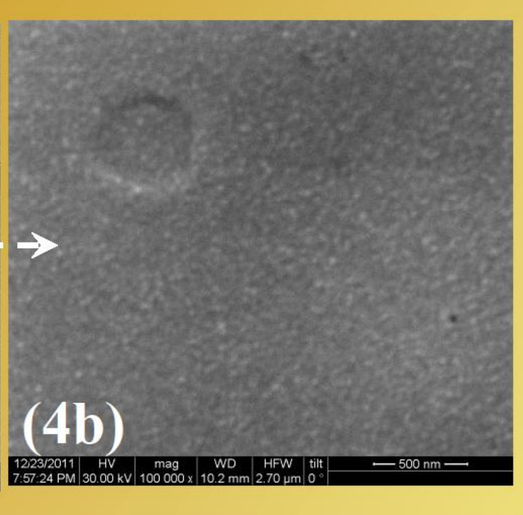
OBJECTIVES: The major factors that motivate and/or promote anaemia in haemodialysis patients (HDp) are (i) reduced production of erythropoietin, (ii) iron deficiency and (iii) reduced lifespan of red blood cells (RBCs). The uraemic milieu has been accused that contributes to the third factor. To investigate the possible biochemical interference of uraemic toxins with the biomolecular constituents of RBCs membrane in the present work we performed biopsy of intact RBCs (iRBCs) of HDp in comparison to healthy donors [1-3].

METHODS: iRBCs of 27 HDp (number of iRBCs=1253) (4-hour dialysis, thrice a week) and of 24 healthy donors (number of iRBCs=1210) were imaged with advanced microscopes. The iRBCs, freshly collected in EDTA tubes, were deposited onto glass slides in single-layered smears with the least processing possible. The smears were surveyed with Atomic-Force Microscope (AFM) (Figures (1a)-(1b)) and Scanning-Electron Microscope (SEM) (Figures (2a)-(2b)). For the study with AFM the smears were absolutely intact, while for SEM a thin overlayer (5-10 nm) of gold was necessarily deposited that however does not obscure the characteristics of the iRBCs membrane. Both AFM and SEM can selectively focus on the iRBCs membrane and reveal information at the nanometer level. Biochemical and hematological data were obtained with the standard clinical techniques.

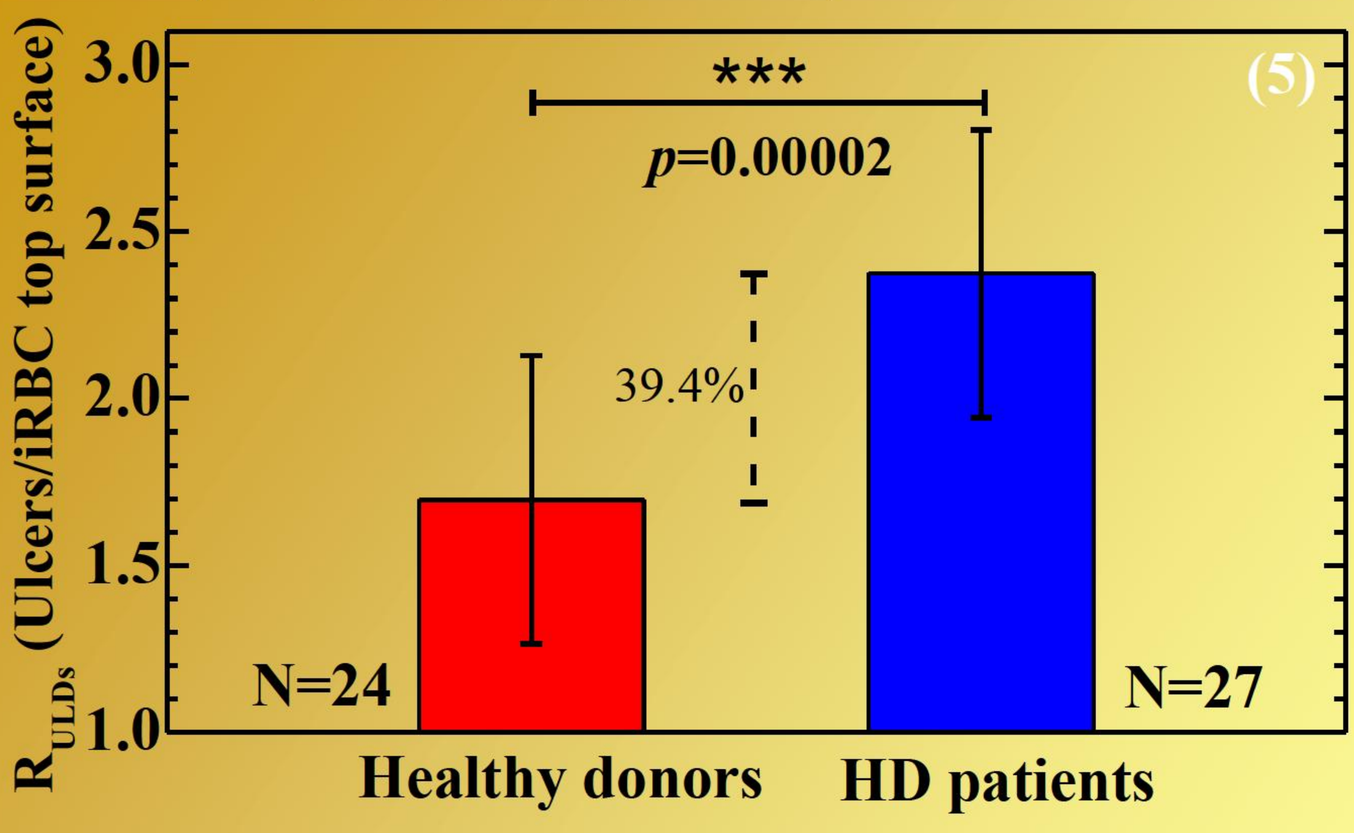








RESULTS: The AFM and SEM data consistently revealed that the iRBCs membrane has ulcer-like deconstructions (ULDs) of almost circular form with typical size 100-2000 nm (Figures (3a)-(3b) and (4a)-(4b), respectively). The ULDs are observed in both the HDp and healthy donors an indication that they possibly relate to physiological aging of RBCs. The population of ULDs per top surface of iRBC is 1.70±0.43 and 2.37±0.57 in the healthy donors and HDp, respectively. The pronounced increase 39.4% observed in the latter group is statistically significant (p=0.00002) (Figure (5)). The direct comparison of the AFM and SEM data with the clinical ones revealed that the population of ULDs correlates with all Ur, Cr, Ca, P and CaxP.



CONCLUSIONS: The observation of ULDs in the iRBCs membrane of both HDp and healthy donors indicates that they relate to physiological aging of RBCs. The statistically significant increase of the ULDs population in the HDp iRBCs proves that the aging of RBCs is accelerated by mechanisms that relate to the underlying disease. Furthermore, the correlation of the ULDs population per iRBC with all basic uremic markers is a proof that uraemia motivates/promotes the structural deconstruction of the RBCs membrane, possibly leading to their premature elimination from the circulation. We believe that this pathway contributes to the worsening of anaemia.

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DOI: 10.3252/pso.eu.51era.2014