

# CYLINDRICAL VESICLES AS DIAGNOSTIC CELLULAR INDICES IN CARDIORENAL SYNDROME TYPES III AND IV: BIOPSY OF PERIPHERAL BLOOD SMEARS WITH THE ATOMIC FORCE AND SCANNING ELECTRON MICROSCOPES

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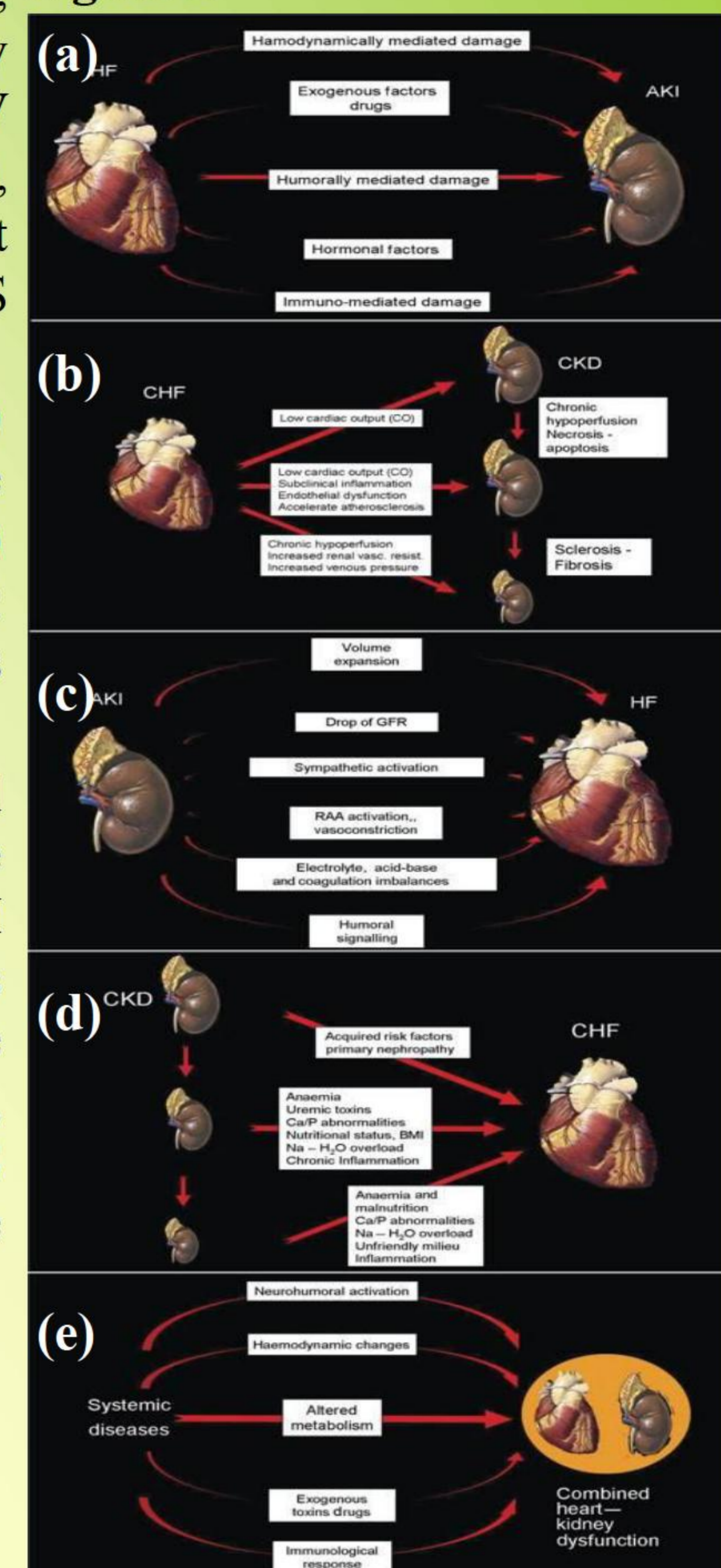
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**INTRODUCTION AND AIMS:** Cardio-Renal Syndrome (CRS) [1] is classified in 5 Types, Figures 1(a)-1(e), depending on the primary impaired organ, Heart (Types I and II) or Kidney (Types III and IV), and the chronic (Types II and IV) or acute (Types I and III) nature. Type V refers to a systemic disease. The biochemical indices (e.g. B-type natriuretic peptide (BNP), neutrophil gelatinase associated lipocalin (NGAL) etc) that document the Type of CRS have not been completely clarified until now. Here we performed biopsy of peripheral blood smears of CRS patients to investigate new cellular diagnostic indices.

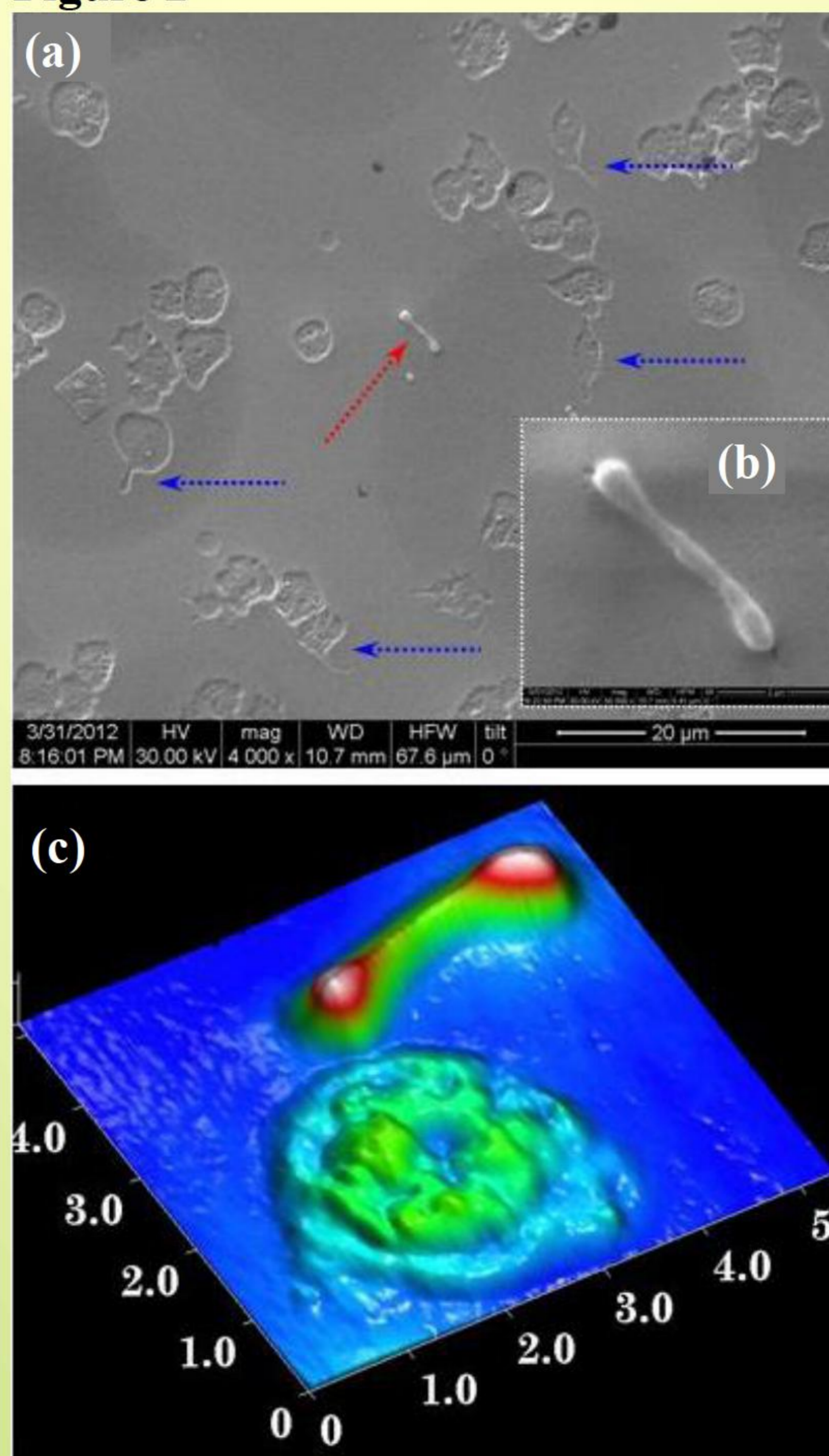
**METHODS:** Smears of peripheral blood were imaged with the Atomic Force Microscope (AFM) and Scanning Electron Microscope (SEM), allowing to obtain quantitative information at the nanometer level (1 nm=10<sup>-9</sup> m) [2-3]. Intact RBCs (iRBCs) (N>1000) and intact platelets (Plts) (N>1000) of patients with CRS (N=5; 2 patients: Types I/II, 2 patients: Types III/IV and 1 patient: Type V) were imaged with AFM and SEM, in comparison with iRBCs and iPlts of healthy donors (N=5). Biochemical and hematological indices were recorded with the standard clinical methods.

**RESULTS:** In all CRS patients were observed micro/nano-metric Vesicles (mnVs) of cylindrical form with spherical ends; see inclined arrow in SEM image, Figure 2(a), inset SEM image, Figure 2(b), and AFM images, Figures 2(c) and 3(a)-3(b). In the 2 CRS patients of Types III/IV the AFM and SEM data revealed an increased population of cylindrical mnVs and activated/abnormal Plts that probably are their parent cells; see horizontal arrows in SEM image, Figure 2(a). In these patients the respective biochemical data evidenced increased levels of uraemic indices, as well as, of BNP and NGAL, Table I. Though the biochemical indices were increased in the other 3 CRS patients of Types I/II/V, no special feature was recorded with the AFM and SEM techniques. None of the above findings was recorded in the 5 healthy donors.

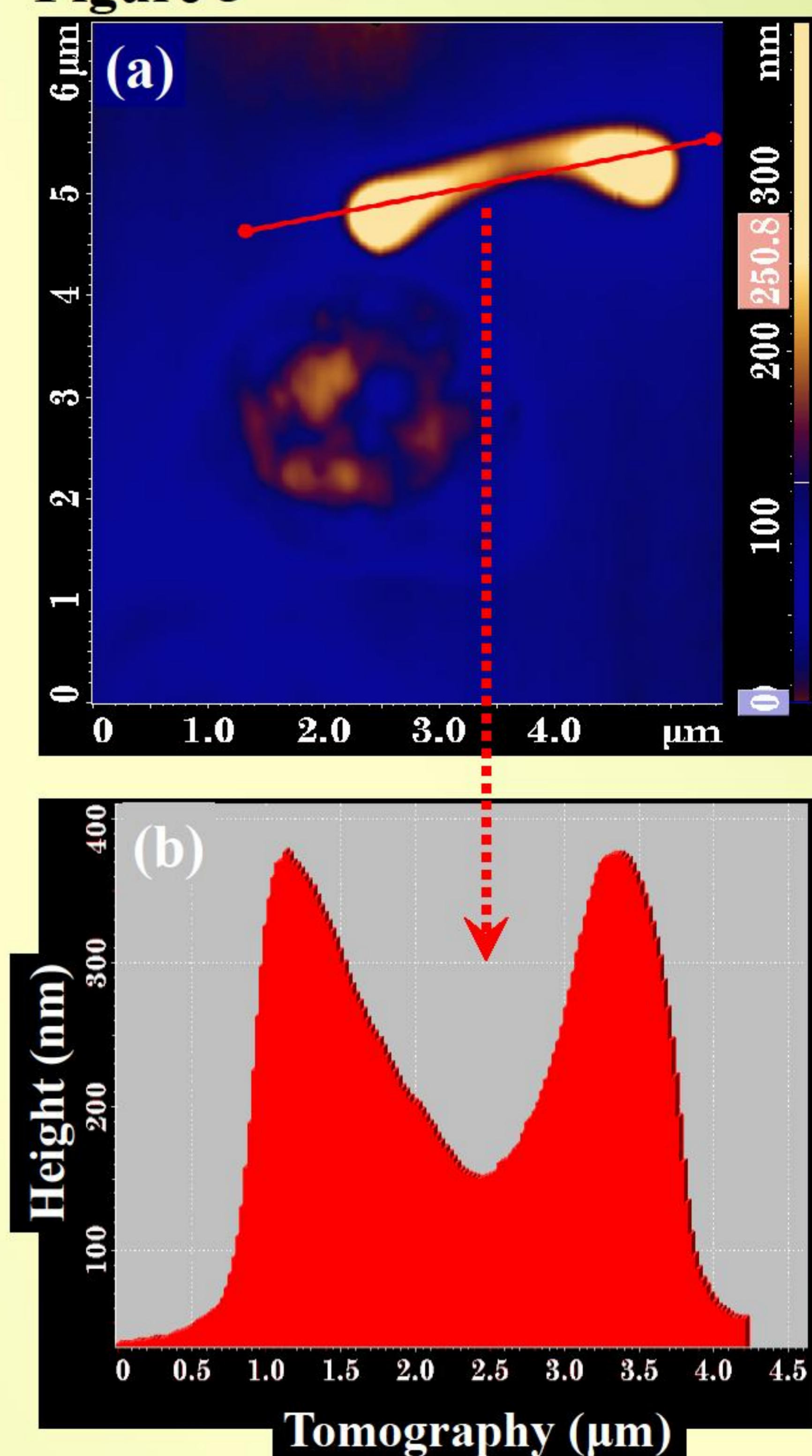
**Figure 1**



**Figure 2**



**Figure 3**



**Table I**

Index	CRS patients (2) Type III and IV	CRS patients (3) Type I, II and V
	mv ± sd (n) *	mv ± sd (n) *
GLU, mg/dl	114.4±25.2 (13)	177.8±145.9 (5)
Ur, mg/dl	228.5±40.0 (14)	120.0±43.3 (5)
Cr, mg/dl	5.0±0.9 (14)	2.6±1.1 (5)
Na, mEq/L	135.7±2.1 (14)	138.4±7.5 (5)
K, mEq/L	4.3±0.5 (14)	4.0±0.7 (5)
Mg, mg/dl	2.9±0.3 (9)	1.7±0.1 (4)
Ca, mg/dl	8.3±0.2 (13)	8.3±0.5 (4)
P, mg/dl	5.1±1.3 (7)	4.5±1.7 (4)
LDH, IU/L	371.5±109.9 (14)	334.0±105.6 (5)
CPK, IU/L	83.1±92.6 (14)	58.0±17.9 (5)
CK-MB, ng/ml	6.6±3.7 (12)	4.5±6.0 (5)
Total proteins, g/dl	5.9±0.6 (5)	6.5±0.8 (4)
ALB, g/dl	2.6±0.4 (5)	2.5±0.2 (4)
CRP, mg/dl	18.0±24.0 (12)	72.0±31.0 (4)
Uric Acid, mg/dl	7.7±1.2 (3)	11.0±0.5 (2)
ALT, IU/L	34.9±52.7 (10)	22.3±11.0 (3)
AST, IU/L	39.3±82.2 (10)	18.7±10.0 (3)
ALP, IU/L	78.0±15.7 (6)	72.7±10.8(3)
Gamma-GT, IU/L	62.1±13.7 (6)	30.7±22.1 (3)
Hb, g/dl	10.5±1.2 (5)	10.4±1.1(5)
Ht, %	32.4±4.1 (5)	31.9±3.1 (5)
WBC, 10 <sup>3</sup> /μl	11.5±7.4 (5)	24.2±33.1 (5)
PLT, 10 <sup>3</sup> /μl	263.2±20.1 (5)	298.6±130.3 (5)
Troponin-I, ng/ml	0.16±0.11 (9)	0.66±1.29 (5)
BNP, pg/ml	1667.0±455.4 (2)	805.4±617.0 (5)
NGAL, ng/ml	1398.5±68.6 (2)	817.3±105.3 (3)
EF, %	45.7±10.1 (3)	42.5±3.5 (2)

\* mean value ± standard deviation (number of experiments)

**CONCLUSIONS:** The CRS of Types III/IV is characterized by cylindrical mnVs that are probably released from Plts. The population of mnVs correlates with the basic uraemic indices and increased levels of BNP and NGAL.

**REFERENCES**

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