Value of biomarkers in evaluation of patients with chronic kidney disease – results from the RENDO and RENART projects

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3 - "Victor Babes" National Institute for Research and Development in Pathology and Biomedical Sciences

RENDO project

Study of circulating endothelial cells and cytokines as early endothelial dysfunction markers in chronic renal failure

RENDO Project Grant no. 42171/2008 - The Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI)

Project Director: Prof. Dr. Mihai Voiculescu

www.rendo.famv.ro

Project Budget: 792.575 RON (2008-2011)

1.	*"Carol Davila" University of Medicine and Pharmacy Bucharest Center of Internal Medicine and Nephrology Fundeni*
2.	*„Victor Babes" National Institute for Research and Development in Pathology and Biomedical Sciences*
3.	*"Prof. Dr. CC. Iliescu" Institute of Cardiovascular Diseases*

RENART project

Proteomics and metabolomics in diagnosis and scoring of vascular calcifications in chronic kidney disease

RENART Project Grant no. 93/2012 - The Executive Agency for Higher Education, Research, Development and Innovation Funding (UEFISCDI)

Project Director: Prof. Dr. Mihai Voiculescu

Project Budget: 3.250.000 RON (2012-2015)

1.	*Fundeni Clinical Institute, Center of Internal Medicine and Nephrology Fundeni*
2.	*„Victor Babes" National Institute for Research and Development in Pathology and Biomedical Sciences*
3.	*"Prof. Dr. CC. Iliescu" Institute of Cardiovascular Diseases*
4.	*Institutul pentru Tehnica de Calcul SA*

Introduction

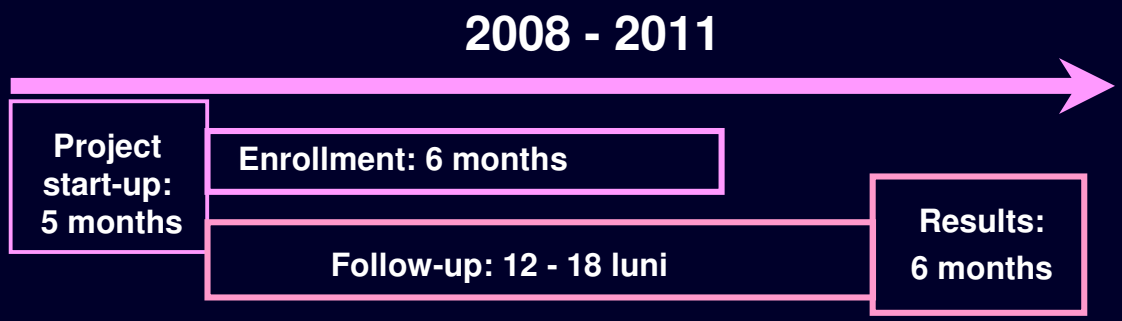
Biomarker *– a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.*

Proteomics *is the systematic study of proteomes, which describes the entire protein content of one or all cells of an organism as well as of bodily fluids such as blood and urine. Proteomics enable the identification and quantification of relevant peptide or protein fragments that might acts as mediators of disease, including cardiovascular disease and CKD.*

Clin Pharmacol Ther 2001; 69: 89-95

RENDO project

The aim of the study is to assess the endothelial dysfunction in chronic kidney disease patients and to evaluate correlations between the surrogate markers of endothelial dysfunction.



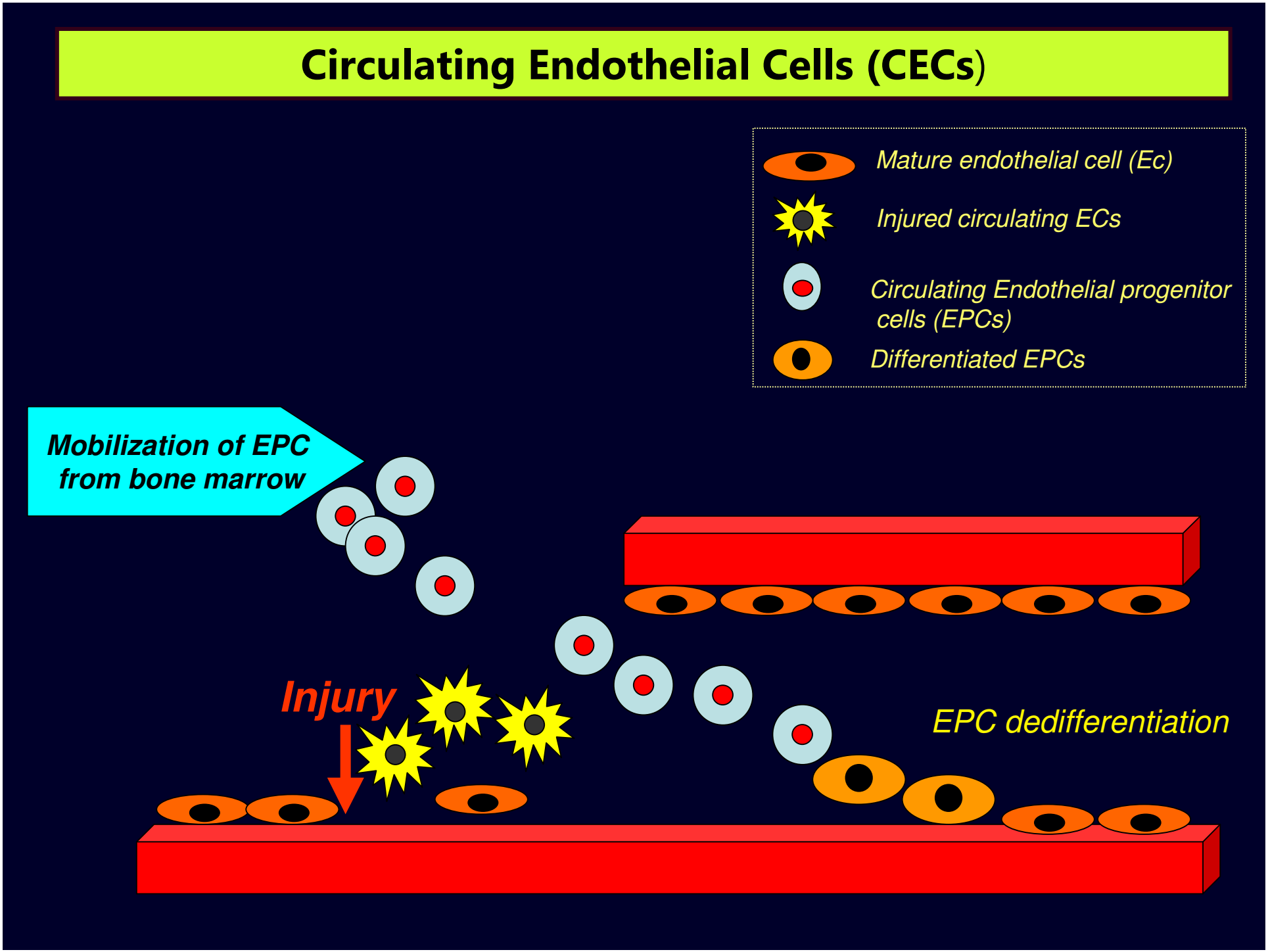
- CECs
- Biomarkers
- FMD
- CMIT
- Arterial stiffness

Assessment of Endothelial Dysfunction

Biomarkers of endothelial dysfunction:

- Intercellular adhesion molecules (ICAM-1)
- Vascular adhesion molecules (VCAM-1)
- E-selectin
- C Reactive Protein
- Interleukin-6
- Interleukin-10
- Tumoral necrosis factor- alpha
- Adiponectine

Circulating Endothelial Cells (CECs)

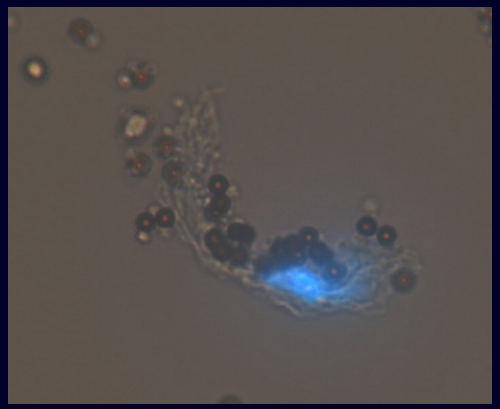
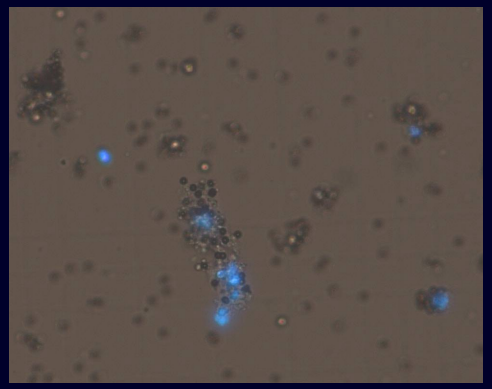
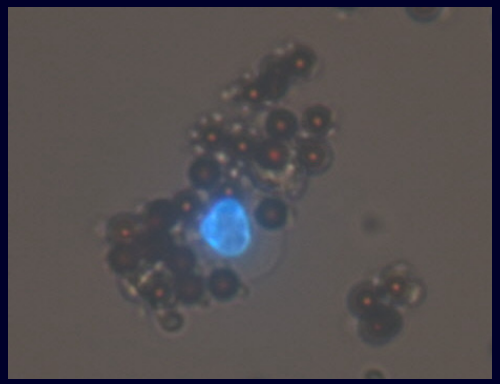


Methods

Measurements of serum biomarkers using xMAP technology (Luminex 200™)

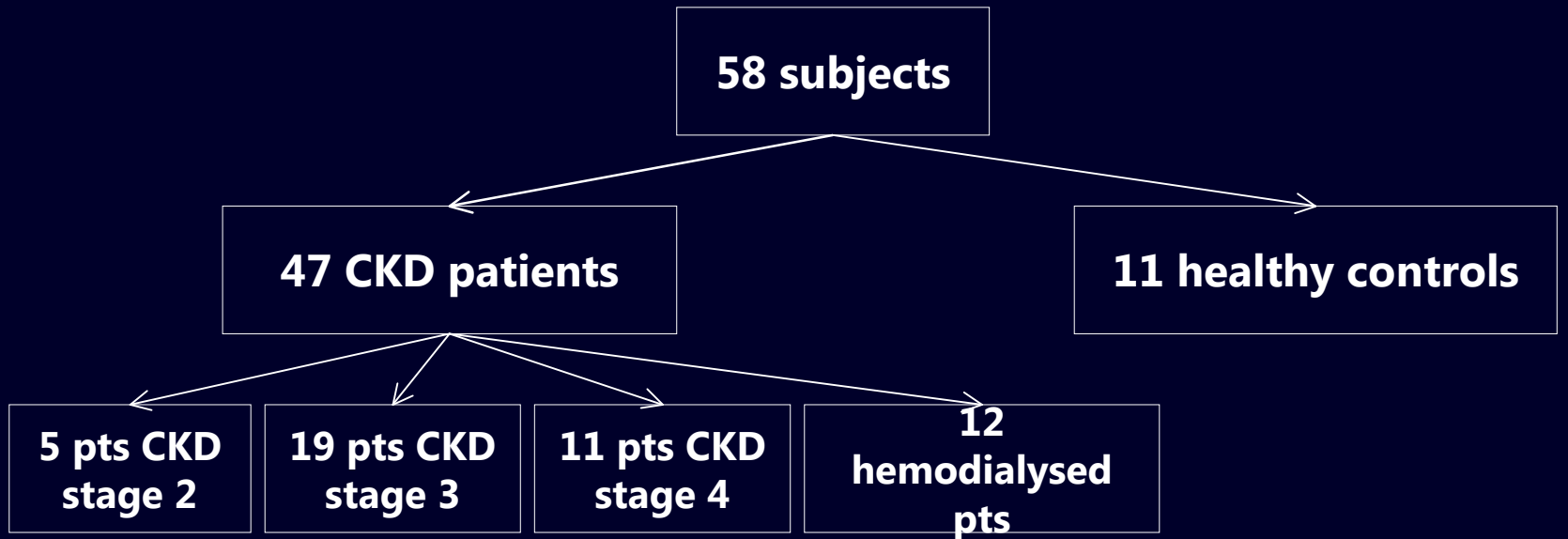
- *Markers of endothelial activation*: vascular cell adhesion molecule - 1 (VCAM-1), E-selectin, intercellular adhesion molecule-1 (ICAM-1)
- *Pro-inflammatory cytokines:* IL-6, TNF-alpha
- *Anti-inflammatory cytokine:* IL-10

- **Measurement of circulating endothelial cells (CECs) by immunomagnetic isolation**

Methods

Prospective study



Etiology of CKD pts in pre-dialysis	*No of pts*
Diabetes mellitus	*12*
Hypertensive nephrosclerosis	*11*
Tubulointerstitial nephritis	*12*

Results

Table. Baseline characteristics and cardiovascular risk factors

Parameter	***CKD stage 2 (n=5)***	***CKD stage 3 (n=17)***	***CKD stage 4 (n=9)***	***Hemodialysis (n=11)***
Age (yr)	65.4 ± 4.9	68.5 ± 6.6	61.3 ± 13.4	57.2 ± 11.9
Male Gender (%)	80	52.6	54.5	83.3
eGFR (ml/min/1.73m^2)	68.1 ± 13.1	42.0 ± 11.0	23.2 ± 6.1	NA
BMI (kg/m2)	31.6 ± 5.2	28.1 ± 4.8	24.5 ± 2.6	28.3 ± 7.7
Hypertension (%)	60	100	100	91.7
Diabetes (%)	60	36.8	18.2	25
Dyslipidemia (%)	60	68.4	85.7	50
Current smoking (%)	20	15.8	18.2	8.3

Results

Subclinical atherosclerosis

-***Carotid wall thickening*** *(IMT > 0.9 mm) or* ***Carotid plaque***
-***Carotid-femoral pulse wave velocity > 12 m/s***
-***Electrocardiographic LVH***

Clinical atherosclerosis

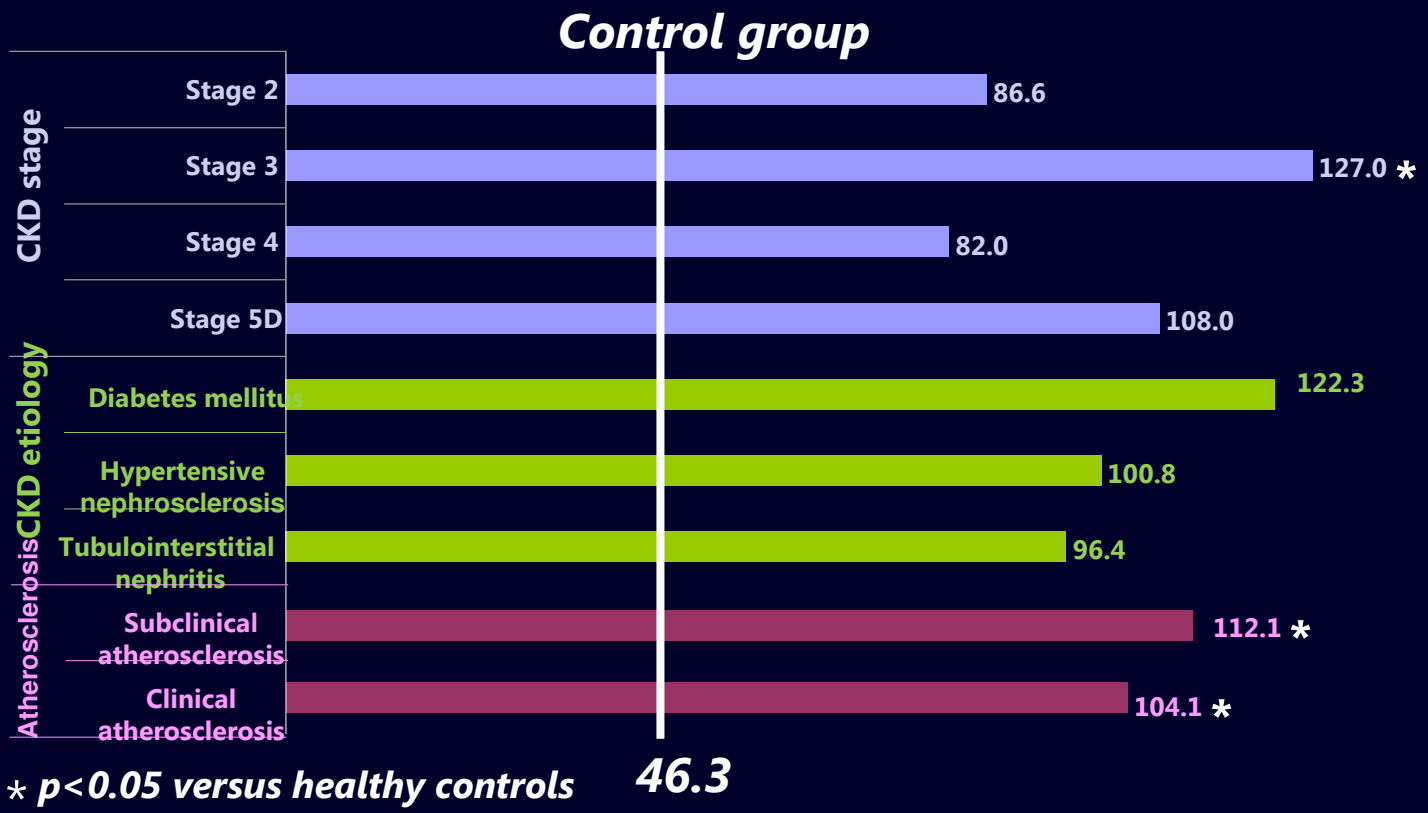
-***Heart disease:*** myocardial infarction, angina, coronary revascularization, chronic heart failure
-***Cerebrovascular disease***: ischemic stroke, cerebral haemorrhage, transient ischemic attack
- ***Peripheral artery disease***

J Am Soc Nephrol 2007;18:2833-2835
European Heart Journal, ESC and ESH Guidelines 2007

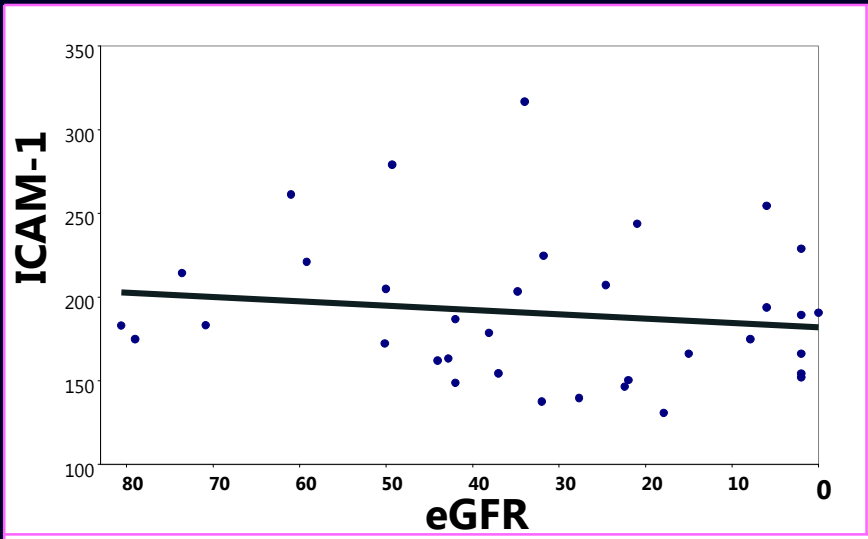
Results

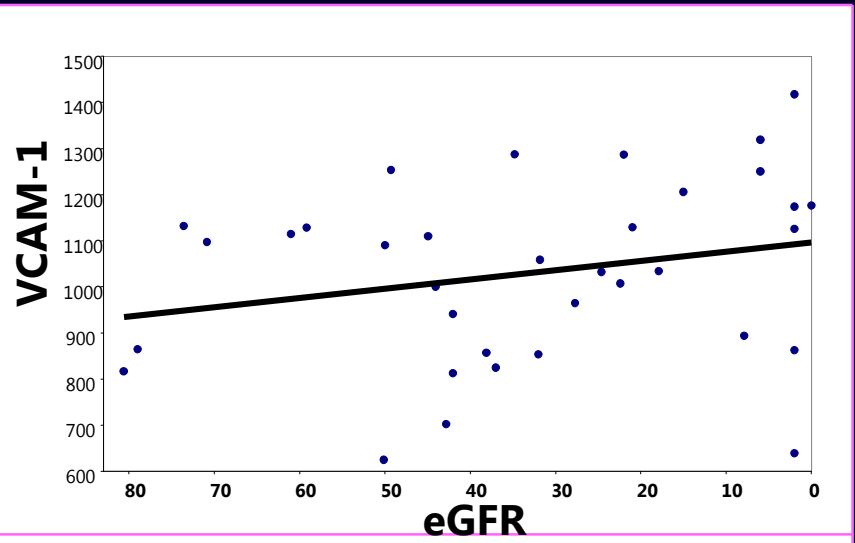
Circulating endothelial cells were significantly increased in CKD patients versus healthy controls.

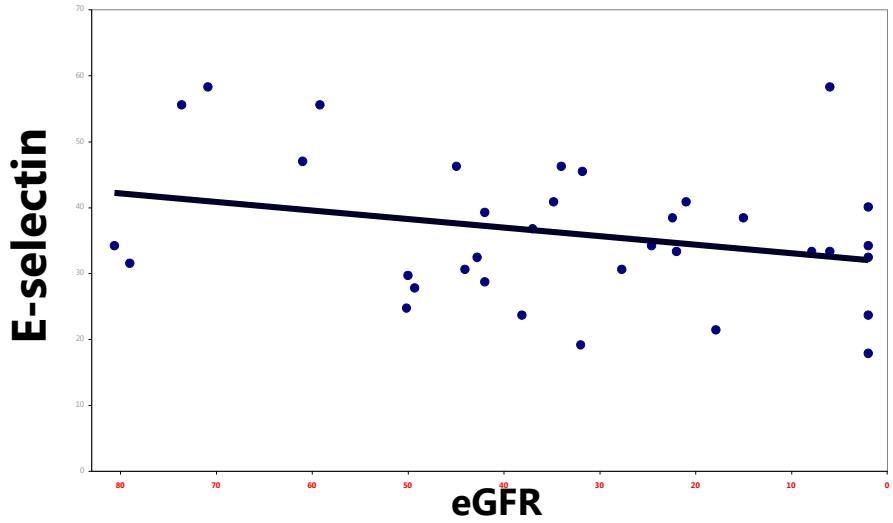
- *Significant differences in mean CECs between different groups:*



Results

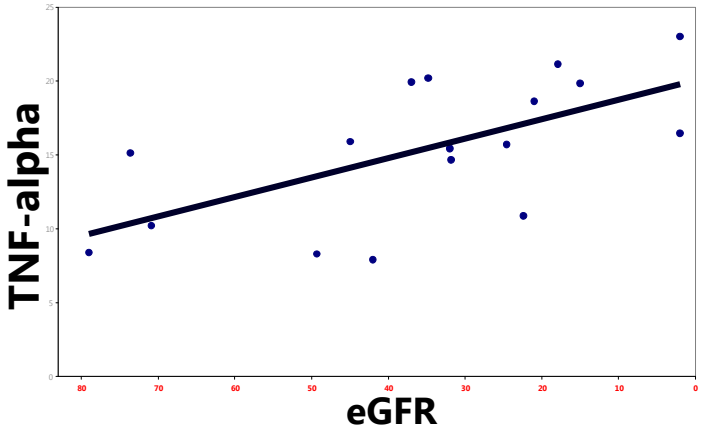

ICAM-1
350
300
250
200
150
100
80
70
60
50
40
30
20
10
0
eGFR

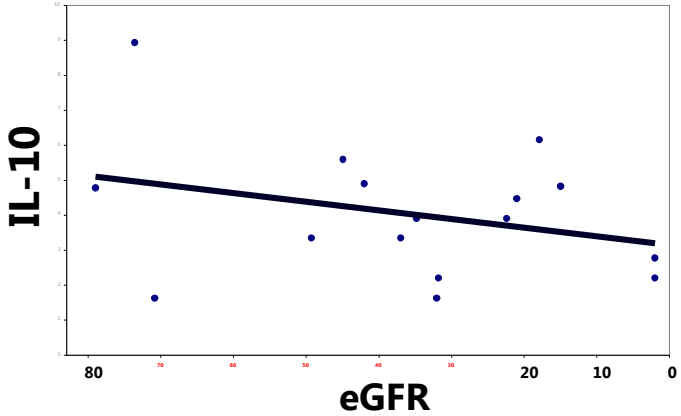

VCAM-1
1500
1400
1300
1200
1100
1000
900
800
700
600
80
70
60
50
40
30
20
10
0
eGFR

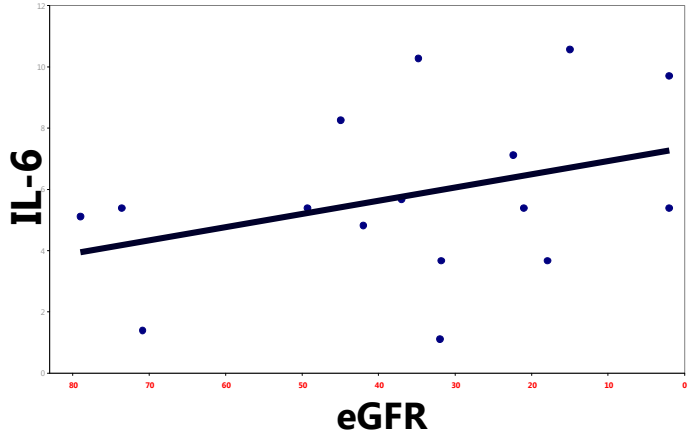

E-selectin
eGFR

Results

TNF – alpha : - **inversely correlated with eGFR (p<0.01)**
- **significant linear relation with eGFR**







Results

	Correlation with eGFR	Significant linear relation with eGFR **
FMD	0.28	No
CECs	0.03	No
PWV	0.01	No
AoSP	-0.03	No
AoPP	-0.19	No
AP	-0.18	No
Aix	**-0.46***	**YES**
IL-6	-0.36	No
IL-10	0.31	No
TNF-alpha	**-0.63***	**YES**
VCAM-1	-0.24	No
ICAM-1	0.14	No
IMT	0.27	No
E-selectin	0.28	No

**) Significant correlation*

***) Based on a level of signification < 0.05 at ANOVA linear regression*

Conclusions

- The study showed that **arterial changes occur early in the course of CKD** as demonstrated by the ***impairment of endothelium-dependent vascular function (FMD), the increase of arterial stiffness and carotid artery intima-media thickness***.

- Measurement of PWV is noninvasive and safe suggesting that attention should be focused on carotid-femoral (aortic) ***PWV as a potential novel biomarker of cardiovascular risk in patients with CKD.***

- ***PWV and CIMT measurement should be recommend for the evaluation of cardiovascular risk particularly in patients in whom target organ damage is not discovered by routine investigations.***

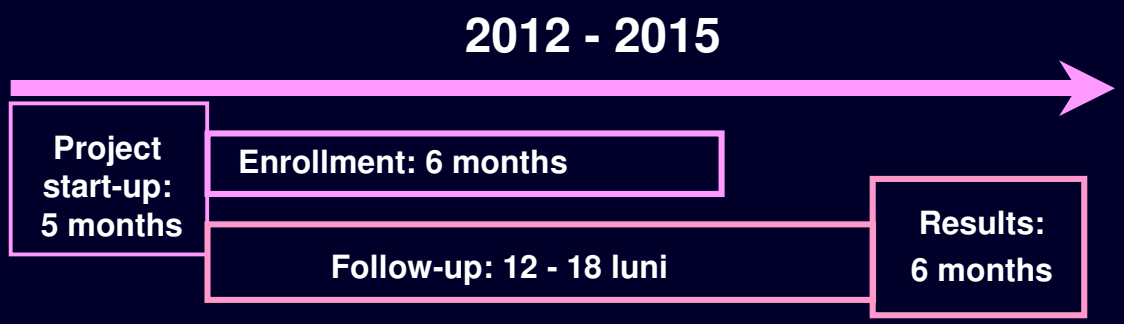
Conclusions

- *In our study was found* ***significant relation between renal function and inflammation,*** *as estimated with* ***TNF-alpha.***

- CECs level correlated with impaired vascular function and was significant
increased in CKD patients.

- Our data underscore that endothelial dysfunction and inflammation were
related with renal function and contribute to cardiovascular mortality in CKD.

RENART project

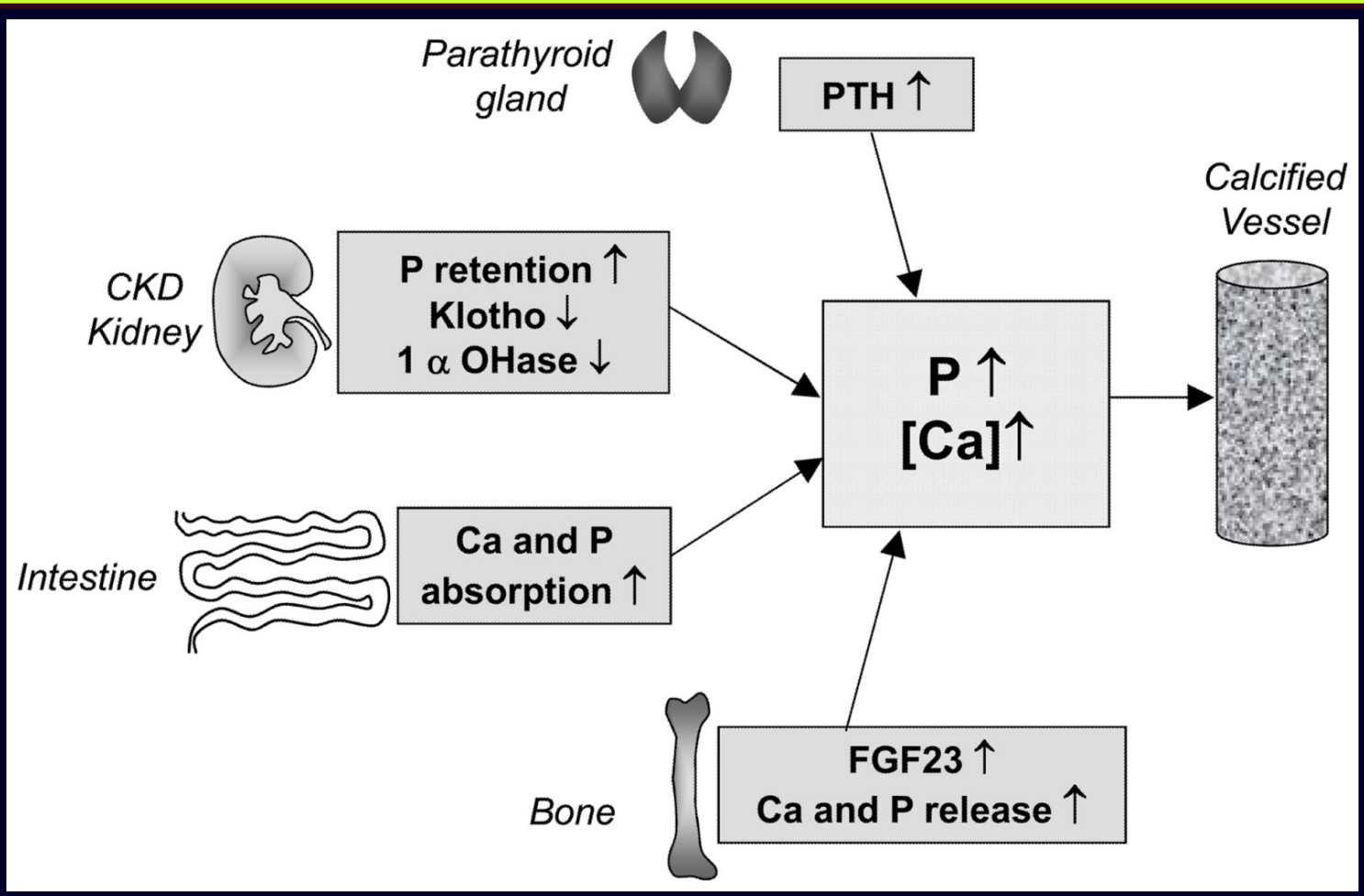


- Biomarkers
- vascular ultrasound
- e-tracking
- Helical computed tomography
- Arterial stiffness

RENART project

- Multiple and different types of observation will be combined: clinical, imaging as well as proteomics, metabolomics
- Development of a mathematical model for vascular calcification assessment
- Development of a computer assisted score for vascular calcification in chronic renal disease – ArterioTest.
- Model validation

RENART project



Factors involved in dysregulation of Ca and P homeostasis in CKD

Circ Res. 2011 September 2; 109(6): 697–711

Biomarkers in CKD - MBD

A multimarker approach

- osteoprotegerin
- Fibroblast growth factor 23 (iFGF23)
- Serum alkaline phosphatase
- serum phosphate
- serum calcium
- intact parathyroid hormone (iPTH)
- Fetuin-A

Endothelial damage is required for development of artery calcification

- hs- C Reactive Protein
- Interleukin-6
- Tumoral necrosis factor- alpha

RENART project – presumed results

The ArterioTest, a computer assisted score for vascular calcification developed within the project will identify and score vascular calcification even from early stages of CKD and will serve to implement guide therapy and to monitor the treatment.