32 Association of hippuric acid, indoxyl sulfate and p-cresyl sulfate with age-related lymphocyte changes in patients on hemodialysis

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Introduction

Alterations of lymphocyte phenotype in CKD and HD

- T and B cell **lymphopenia**
- **reduction** in naïve and early differentiated lymphocytes
- **accumulation** of advanced differentiated subsets[1,2]

Gut dysbiosis (gut microbiome alterations)

- promotes **dysregulation** of immune system:
  - abnormal activation of immune cells
  - overproduction of inflammatory factors[3]

Uremic toxins classification by EUTox

- free water-soluble low-MW solutes (< 500 Da)
- protein-bound solutes
- middle MW molecules (> 500 Da)[4,5]

CKD: chronic kidney disease; HD: hemodialysis;
EUTox: European Uremic Toxin Work Group; MW: molecular weight

[1] Xiang et al., 2020. *BMC Nephrol.*, 21(1):271.
[2] Stangou et al., 2022. *Front Med (Lausanne).*, 9:874581.
[3] Chi et al., 2021. *J Immunol Res.*, 2021:5516035.
[4] Vanholder et al., 2003. *Kidney Int.*, 63(5):1934-1943.
[5] Rosner et al., 2021. *Clin J Am Soc Nephrol.*, 16(12):1918-1928.

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Study aims

Protein-bound uremic toxins MW:
- indoxyl sulfate (IxS): 212 Da
- p-cresyl sulfate (pCS): 31 Da
- hippuric acid (HA): 179 Da[6,7]

Measurement of PBUT levels

Comparison of PBUT levels in patients on HD and control group

Association between PBUT levels and lymphocyte alterations

Aims

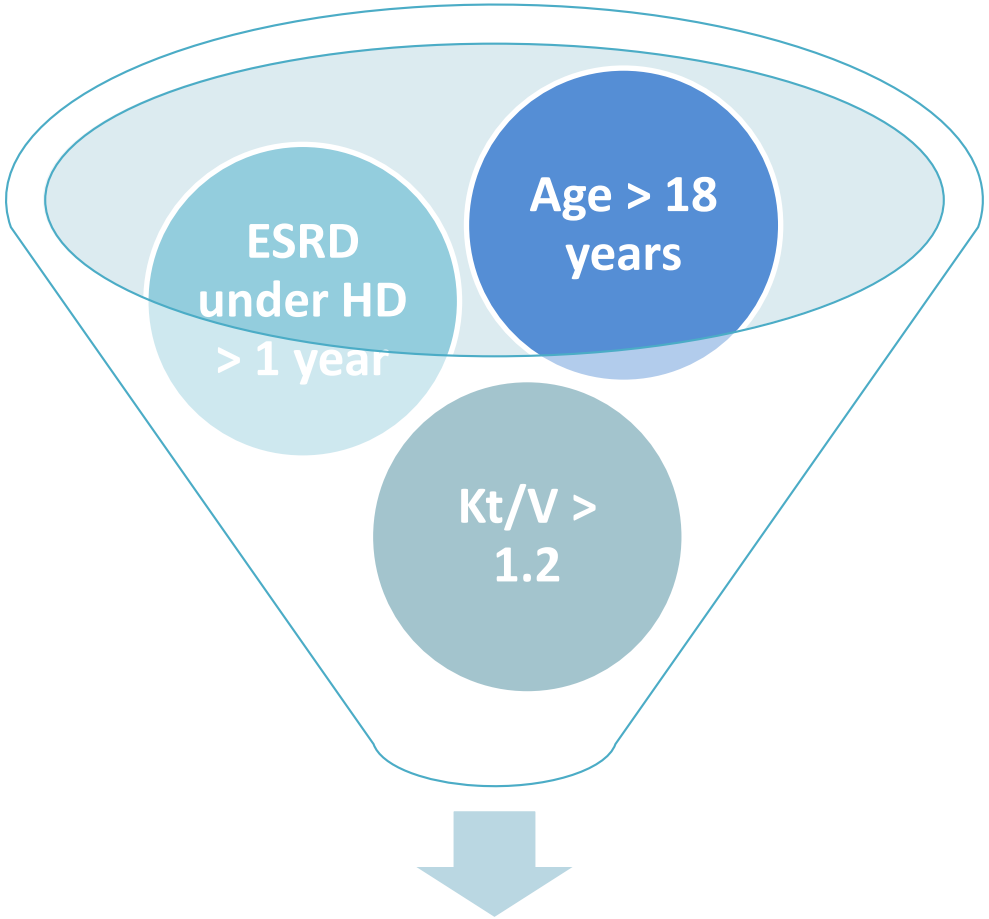
PBUT: protein-bound uremic toxins;
MW: molecular weight; HD: hemodialysis

[6] Duranton et al., 2012. *J Am Soc Nephrol*., 23(7):1258-1270.
[7] Brunet et al., 2003. *Adv Ren Replace Ther*., 10(4):310-320.

Study	Site	Measurement	Evaluation	Parameters
Cross sectional	Thessaloniki	PBUT by UPLC	Lymphocyte surface receptors of T and B cells by flow cytometry	Demographic Dialysis Laboratory

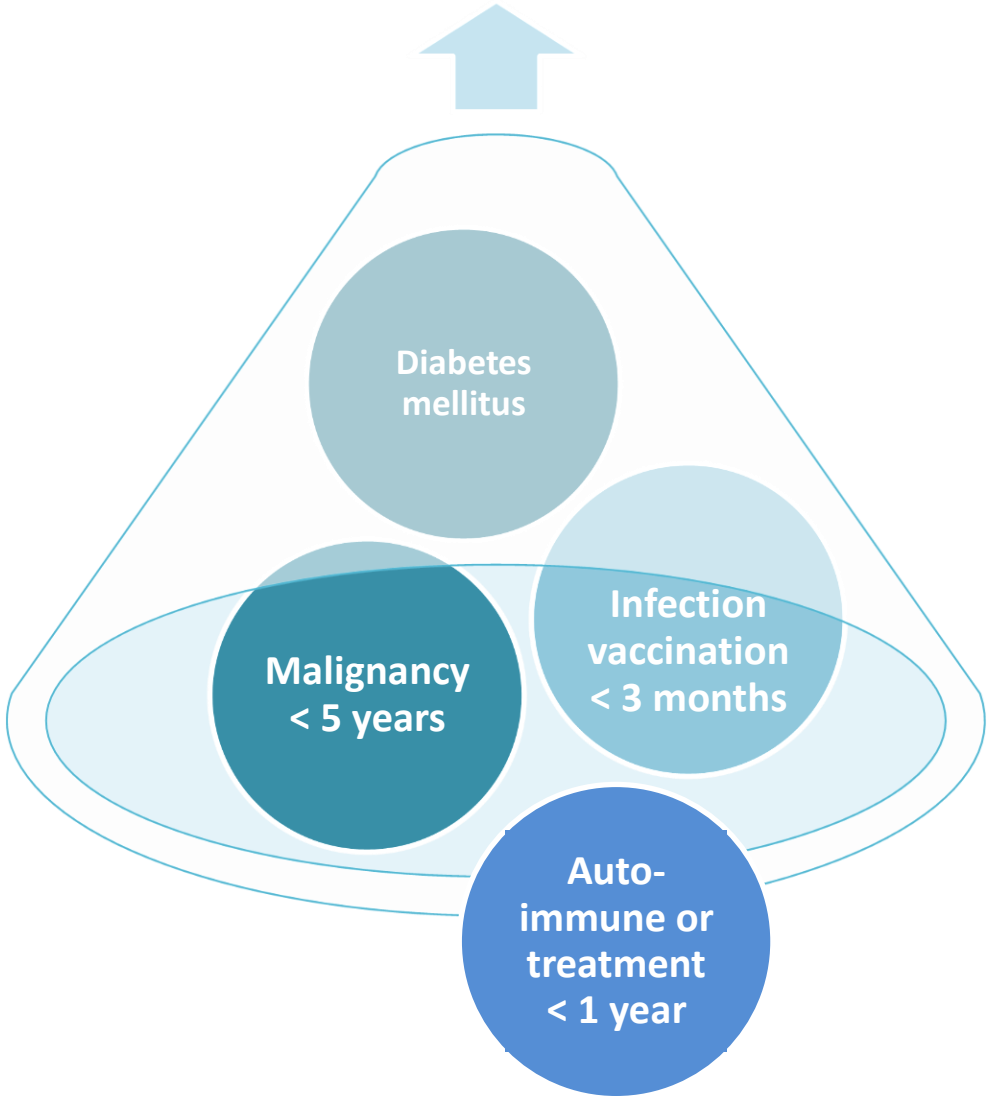
Methods

PBUT: protein-bound uremic toxins;
UPLC: ultra-performance liquid chromatography



Inclusion criteria

Exclusion criteria

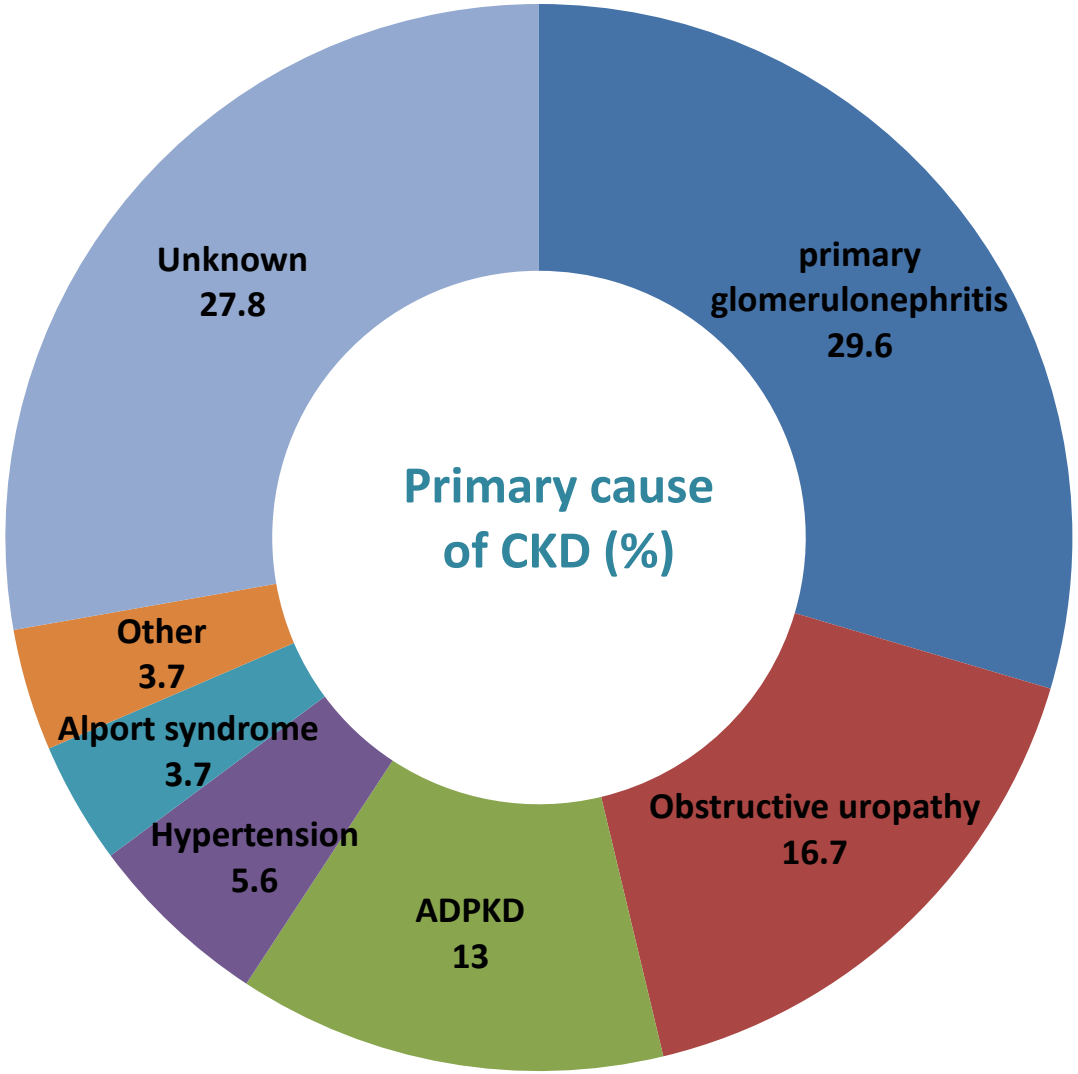


ESRD: end stage renal disease;
HD: hemodialysis

Patients on HD data (n=54)

Parameter	range or SD
Age (years)	**51.3**±16.9
Sex (female/male)	23/31
Body mass index (kg/m^2)	24.5±3.6
Residual kidney function (%)	38.9
Arterial hypertension (%)	66.7
Dyslipidemia (%)	31.5
Dialysis vintage (months)	**67** (20.7-95.2)
HD / Hemodiafiltration	30/24

vs 31 healthy controls
15 females/16 males
Age **51.32**±17.23 years



HD: hemodialysis; CKD: chronic kidney disease;
ADPKD: autosomal dominant polycystic kidney disease

Patient's laboratory data

Parameter	range or SD
White blood cells (cells/μL)	7100 (5500-8325)
Neutrophils (cells/μL)	4550 (3475-5500)
Lymphocytes (cells/μL)	1400 (1175-1800)
Monocytes (cells/μL)	602.4 (538.2-758.4)
Hematocrit (%)	36.1±3.1
Hemoglobin (g/dL)	11.8±0.9
Platelets (10^3/μL)	227 (184.2-265)

Parameter	range or SD
Urea (mg/dL)	**127** (111.5-151.5)
Creatinine (mg/dL)	**9.4** (7.3-10.9)
Calcium (mg/dL)	9.1 (8.8-9.3)
Phosphorus (mg/dL)	4.3 (3.7-5)
Parathyroid hormone (pg/mL)	206.5 (106.2-370.7)
Cholesterol (mg/dL)	156.5 (122.5-172.5)
Triglycerides (mg/dL)	129 (84.7-163.7)
Ferritin (ng/mL)	301 (158-440)
Albumin (g/dL)	4.1 (3.9-4.3)
Lactate dehydrogenase (IU/L)	164 (145-187.2)
C reactive protein (mg/L)	2.3 (1.4-4.2)

Results - PBUT plasma levels

A **higher** concentration of **total** and **free** levels of **PBUT** was observed in patients on HD, in comparison to control group (**p<0.001**)

PBUT	Patients (mg/dL)	Control group (mg/dL)
Total HA	3.05 (1.66-5.37)	0.102 (0.04-0.2)
Free HA	1.482 (0.7-2.8)	0.029 (0.03-0.04)
Total IxS	2.207 (1.27-3.34)	0.063 (0.04-0.09)
Free IxS	0.146 (0.09-0.27)	0.0004 (0.0004-0.0004)
Total pCS	1.248 (0.84-1.66)	0.066 (0.04-0.13)
Free pCS	0.089 (0.06-0.13)	0.004 (0.004-0.005)

PBUT: protein-bound uremic toxins; HD: hemodialysis;
HA: hippuric acid; IxS: indoxyl sulfate; pCS: p-cresyl sulfate

PBUT and immunosenescence

Naïve and **less differentiated T** cells correlate with **total** and **free HA** levels. **Naïve** and **non-switched memory B** cells had negative association with **HA** and **free IxS**

	Total HA		Free HA		Free IxS	
	r	p	r	p	r	p
CD4+CD45RA+CD57-	-0.3	0.03	-0.3	0.02	NS	NS
CD4+CD28+CD57-	-0.3	0.05	-0.3	0.03	NS	NS
CD8+CD28+CD57-	-0.3	0.01	-0.3	0.01	NS	NS
CD19+IgD+CD27-	-0.3	0.04	-0.3	0.03	-0.3	0.01
CD19+IgD+CD27+	NS	NS	NS	NS	-0.3	0.01

PBUT: protein-bound uremic toxins;
HA: hippuric acid; IxS: indoxyl sulfate;
NS: not significant

PBUT and immunoexhaustion

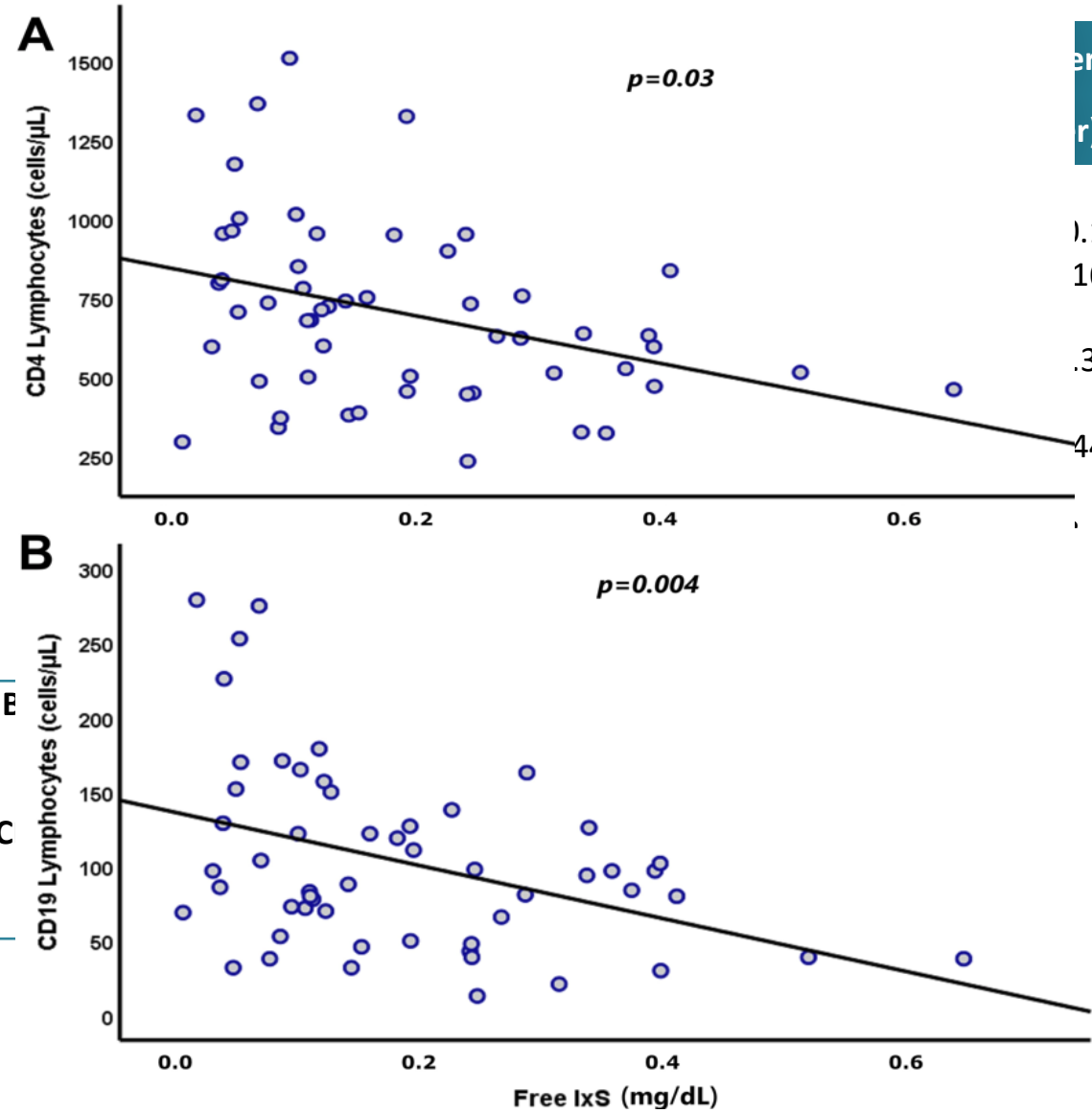
Exhausted CD4 and further **divided** lymphocytes correlated with **total** and **free pCS**

	Total pCS		Free pCS	
	r	**p**	**r**	**p**
CD4+PD1+	0.3	0.02	0.3	0.01
CD4+CD45RA+PD1+	0.3	0.04	0.3	0.045

PBUT: protein-bound uremic toxins;
pCS: p-cresyl sulfate

Multivariate analysis

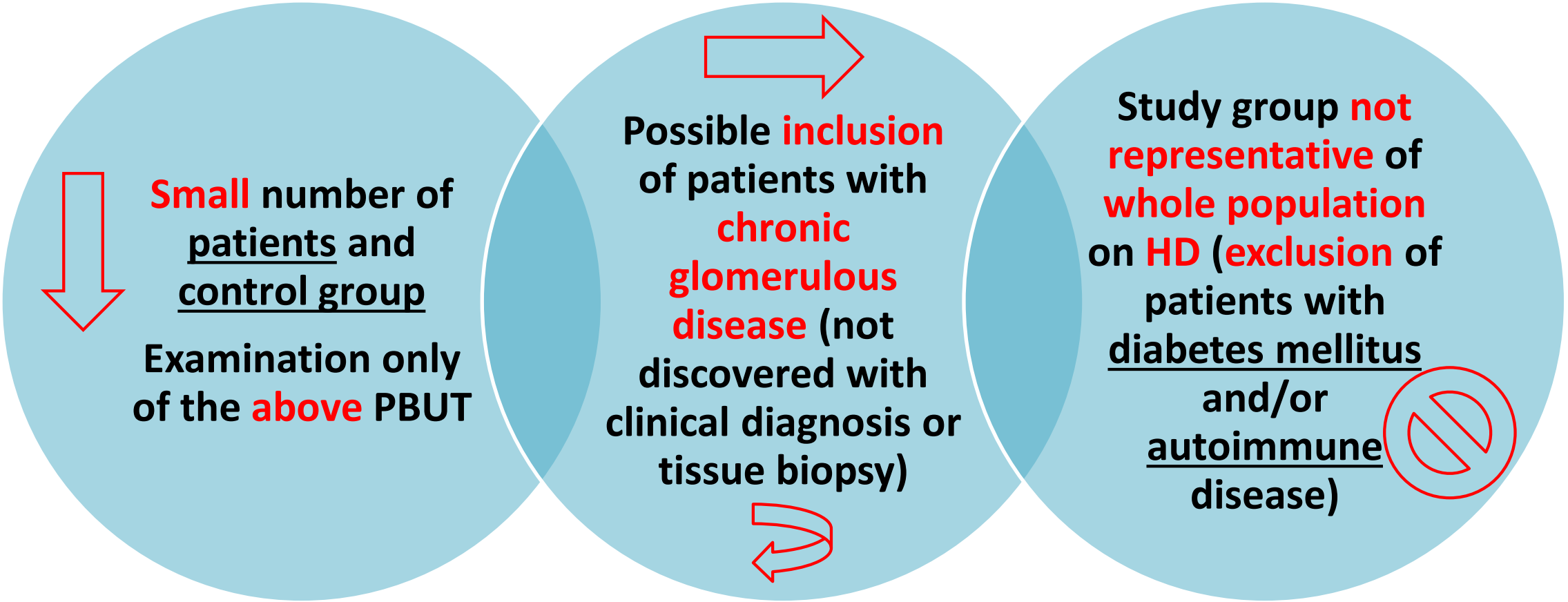
Multivariate analysis showed that **age** and **IxS** had independent role in the **reduction** of **CD4** (**A**) and **B** (**B**) lymphocytes and their **naïve** and **early differentiated** subsets. **PCS** was the leading coefficient related with **exhausted CD4+PD1+** cells



	erval r)	p
	.17	0.007
	16	0.04
	.3	0.001
	44	0.04
		0.02
		0.009
		0.04
	2	0.001
		<0.0001
		0.004
		0.001

IxS: indoxyl sulfate;
pCS: p-cresyl sulfate

Study limitations



PBUT: protein-bound uremic toxins;
HD: hemodialysis

Conclusions

Higher plasma levels of **HA**, **IxS**, **pCS** were noticed in patients on HD vs control group

HA and **IxS** correlated with **immunosenescent** changes in patients on HD

pCS correlated with **immunoexhausted** changes in patients on HD

HA: hippuric acid; IxS: indoxyl sulfate; pCS: p-cresyl sulfate;
HD: hemodialysis

Thank you

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