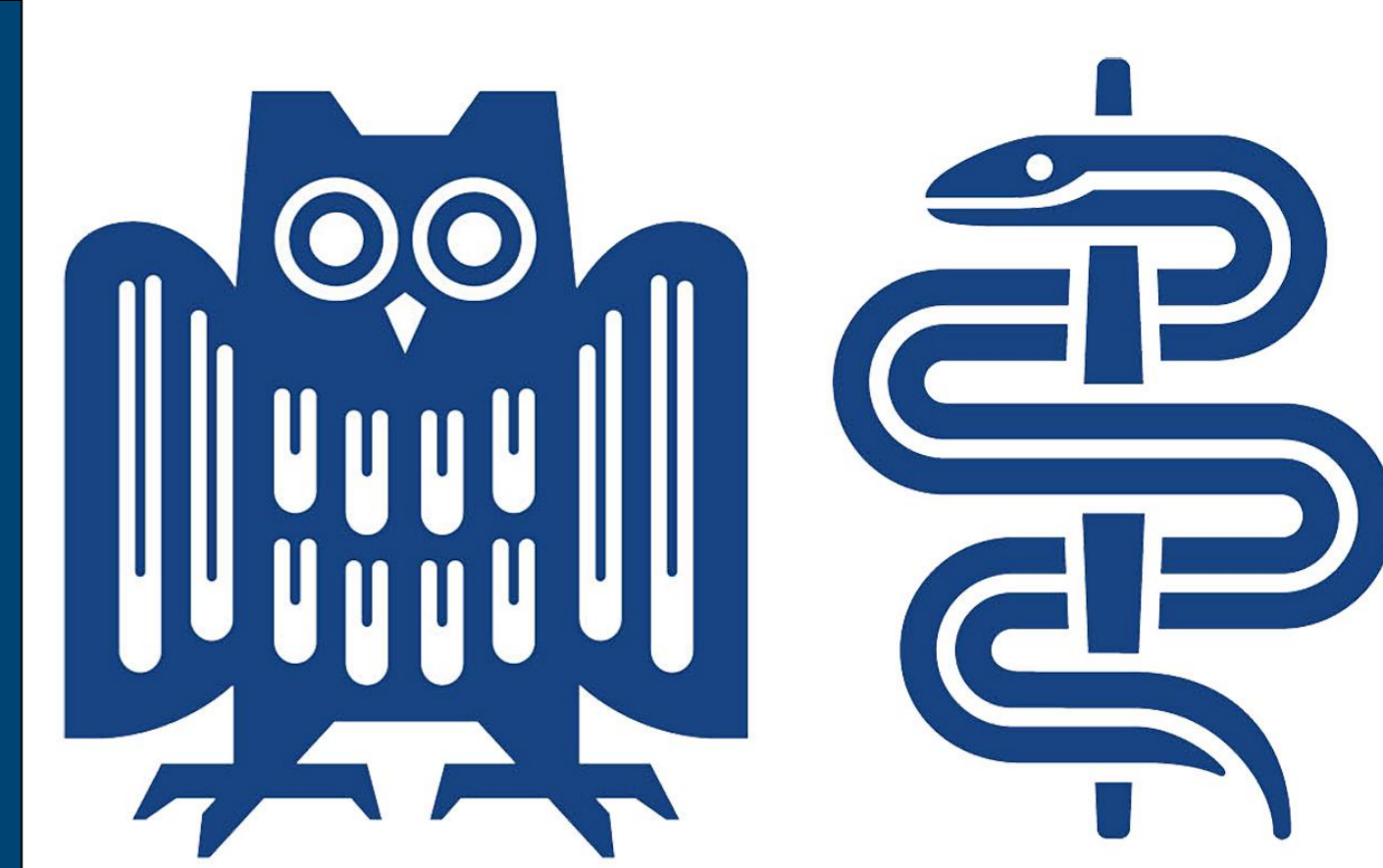


# Gating strategy for the identification of intermediate monocytes in patients with chronic kidney disease



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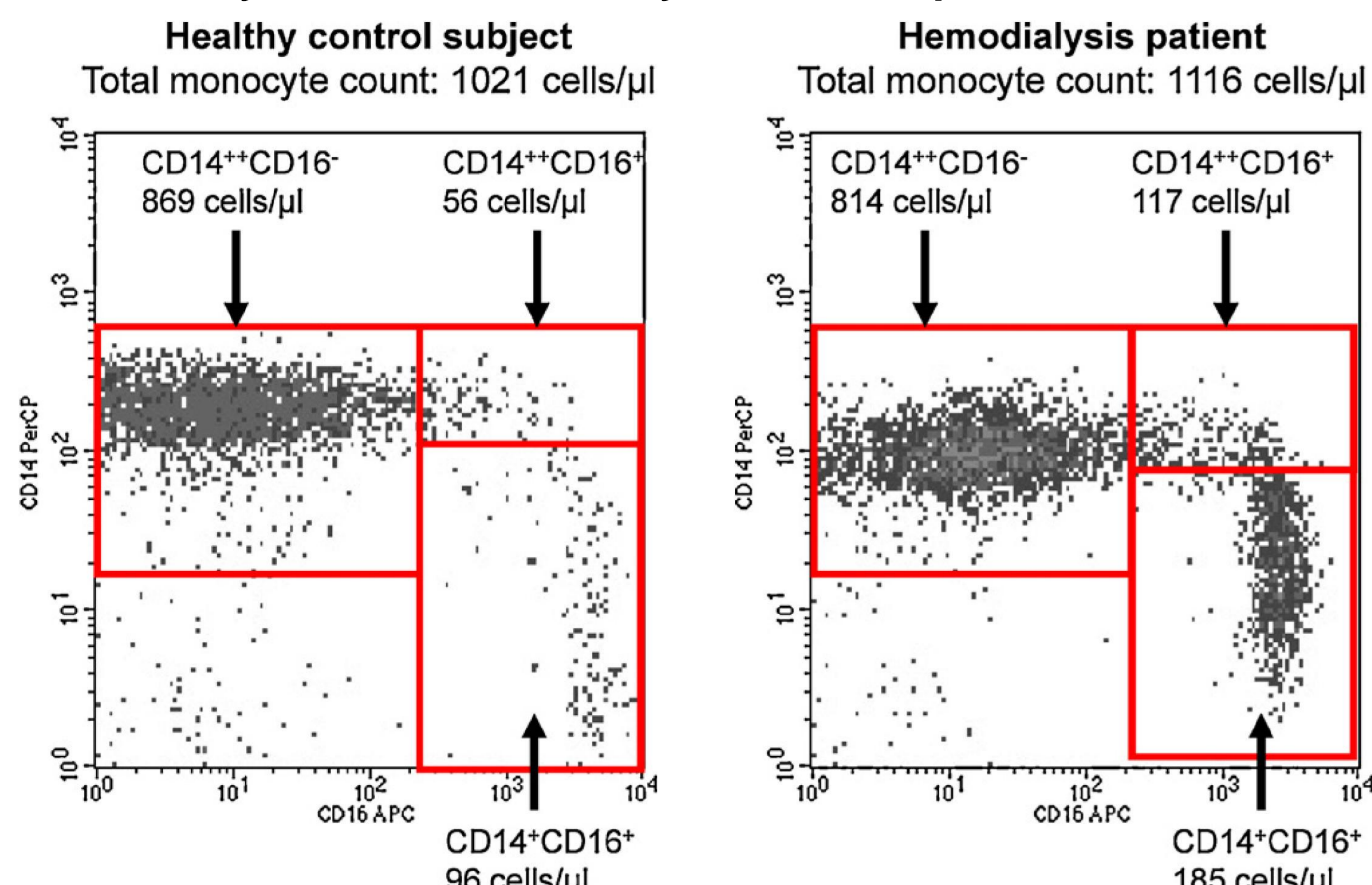
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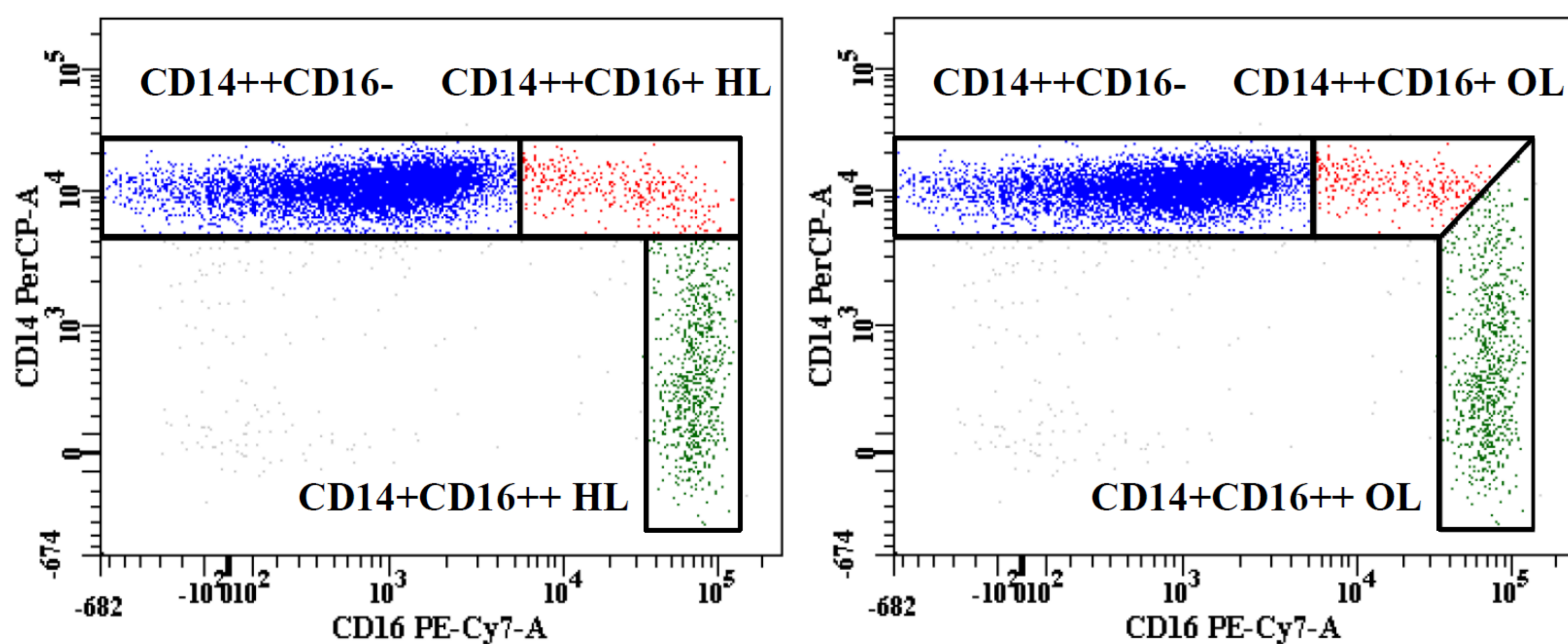
## Introduction

Monocytes are a heterogeneous cell population consisting of three different subsets: classical CD14<sup>++</sup>CD16<sup>-</sup>, intermediate CD14<sup>++</sup>CD16<sup>+</sup> and nonclassical CD14<sup>+</sup>CD16<sup>++</sup> monocytes. Intermediate monocytes have been identified as independent predictors of cardiovascular events among patients with chronic kidney disease (CKD). Importantly, correct enumeration of cell counts of monocyte subsets necessitates well-defined gating strategies. Two strategies have been suggested for the dissection of intermediate from nonclassical monocytes: dissection by an oblique line or dissection by a vertical line (see Figure). Here we compared the prognostic implications of both gating strategies in a well defined cohort of patients with CKD.

### Monocyte subsets: healthy control vs patient with CKD



### Different gating strategies: horizontal (HL) vs oblique (OL) gating



## Results

### Baseline characteristics

	Total cohort (n = 416)	No CVE (n = 310)	CVE (n = 106)	P-Value
Age (years)	65.1±12.6	63.3±13.0	70.5±9.4	<0.001
Sex (male)	248 (60%)	175 (57%)	73 (69%)	0.029
Diabetes mellitus (Y)	161 (39%)	104 (34%)	57 (54%)	<0.001
Smoking (Y)	41 (10%)	29 (9%)	12 (11%)	0.338
Prevalent CVD (Y)	129 (31%)	63 (20%)	66 (62%)	<0.001
BMI (kg/m <sup>2</sup> )	30.2±5.5	30.3±5.5	29.8±5.5	0.438
BP systolic (mmHg)	154±25	154±24	154±27	0.908
BP diastolic (mmHg)	87±13	88±12	82±13	<0.001
BP mean (mmHg)	109±15	110±14	106±16	0.021
eGFR (ml/min/1.73m <sup>2</sup> )	45.2±15.9	47.7±15.6	37.7±14.3	<0.001
UAE (mg/g creatinine)	36 (8-190)	27 (7-156)	71 (24-316)	0.125
CRP (mg/l)	2.7 [1.2-5.4]	2.4 [1.1-4.6]	4.1 [1.7-9.7]	0.001
Total cholesterol (mg/dl)	193±43	198±42	177±41	<0.001
LDL-C (mg/dl)	115±36	118±36	106±33	<0.001
HDL-C (mg/dl)	47 [39-61]	49 [41-63]	44 [36-55]	0.009
Triglycerides (mg/dl)	135 [97-192]	136 [97-207]	132 [96-164]	0.270
Apo A-I (mg/dl)	161 [142-184]	166 [146-188]	148 [134-167]	<0.001
Total monocytes (cells/μl)	562±204	536±193	638±218	<0.001
Classical monocytes (cells/μl)	464±175	444±166	521±186	<0.001
Intermediate monocytes HL (cells/μl)	35±22	31±17	45±28	<0.001
Intermediate monocytes OL (cells/μl)	27±17	24±15	36±21	<0.001
Nonclassical monocytes HL (cells/μl)	64±32	61±30	71±38	0.014
Nonclassical monocytes OL (cells/μl)	70±36	66±32	79±44	0.006

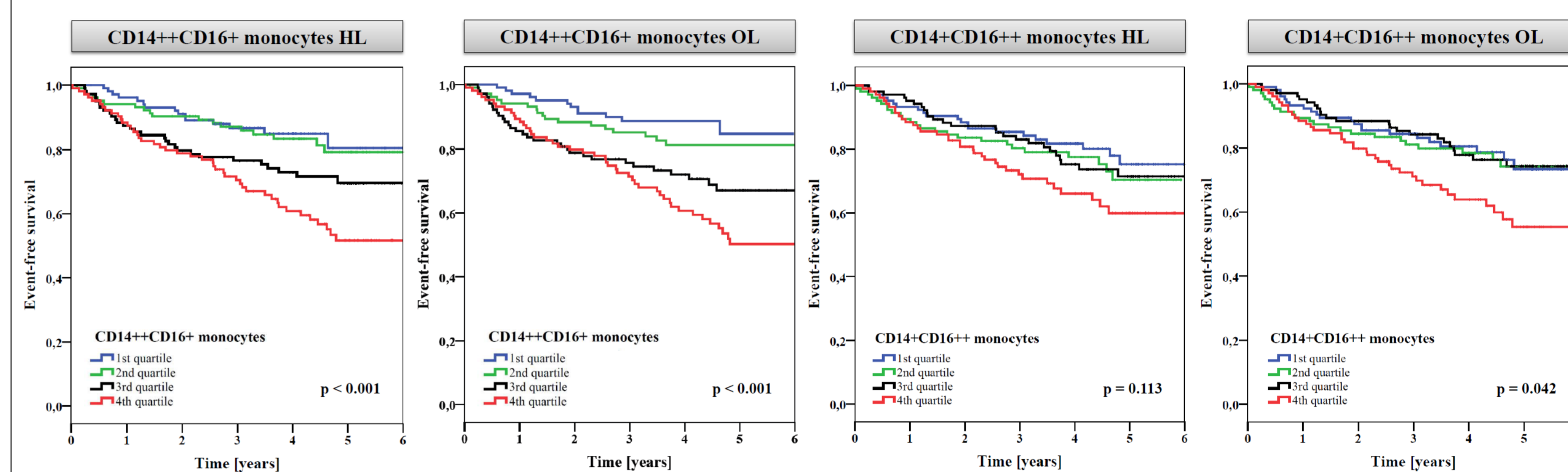
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## Results

### Cross-sectional analysis

Variable	Intermediate monocytes			Nonclassical monocytes						
	HL	OL	HS vs OL	HL	OL	HL vs OL				
Age	0.124	0.012	0.125	0.011	0.988	0.102	0.037	0.107	0.030	0.942
BMI	0.053	0.279	0.043	0.379	0.886	0.153	0.002	0.149	0.002	0.953
Hip circumference	0.001	0.980	-0.010	0.835	0.875	0.104	0.035	0.100	0.041	0.954
Waist circumference	0.009	0.860	-0.002	0.971	0.875	0.173	<0.001	0.162	0.001	0.871
Systolic BP	-0.082	0.095	-0.085	0.084	0.965	-0.065	0.189	-0.065	0.183	1.000
Diastolic BP	-0.144	0.003	-0.147	0.003	0.965	-0.109	0.026	-0.112	0.022	0.965
Mean BP	-0.129	0.009	-0.132	0.007	0.965	-0.099	0.045	-0.101	0.040	0.977
Creatinine	0.157	0.001	0.152	0.002	0.941	0.053	0.282	0.067	0.174	0.840
UAE	0.046	0.349	0.044	0.369	0.977	-0.056	0.257	-0.045	0.362	0.874
eGFR	-0.147	0.003	-0.144	0.003	0.965	-0.044	0.373	-0.057	0.247	0.851
CRP	0.297	<0.001	0.269	<0.001	0.662	0.189	<0.001	0.217	<0.001	0.675
Total cholesterol	-0.035	0.483	-0.040	0.420	0.943	-0.036	0.463	-0.033	0.503	0.966
LDL cholesterol	0.025	0.618	0.019	0.693	0.931	-0.010	0.842	-0.002	0.960	0.909
HDL cholesterol	-0.155	0.002	-0.155	0.002	1.000	-0.125	0.011	-0.130	0.008	0.942
Triglycerides	0.018	0.710	0.016	0.747	0.977	0.032	0.510	0.032	0.512	1.000
Leukocytes	0.323	<0.001	0.319	<0.001	0.949	0.304	<0.001	0.307	<0.001	0.962

### Univariate Kaplan-Meier analysis

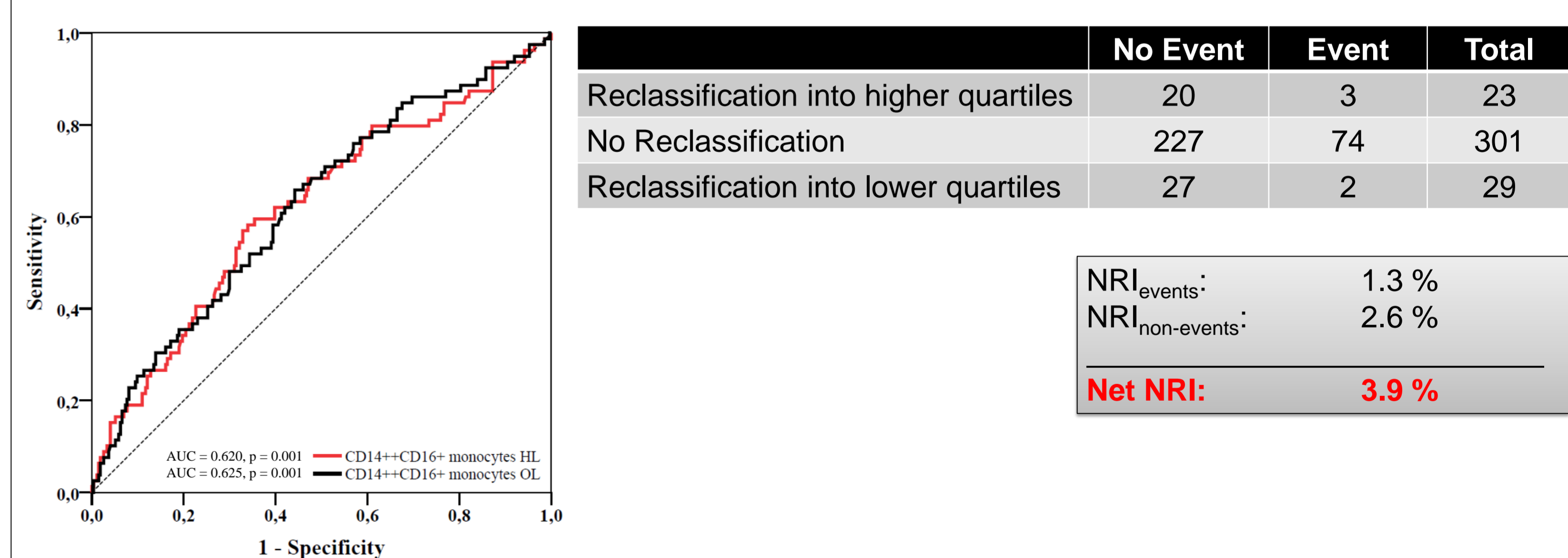


### Cox Regression analysis

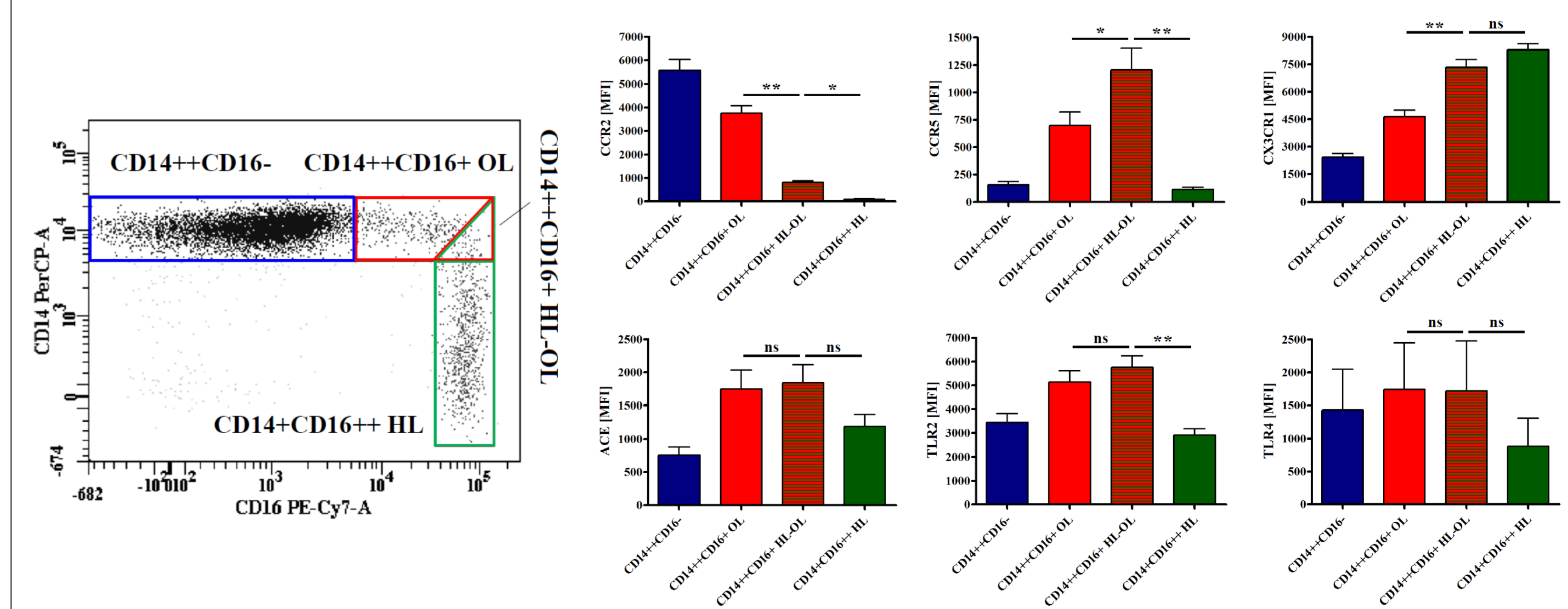
	HR	CI	p
CD14 <sup>++</sup> CD16 <sup>+</sup> monocytes HL (cells/μl)	1.013	1.006-1.020	<0.001
CD14 <sup>++</sup> CD16 <sup>+</sup> monocytes OL (cells/μl)	1.015	1.006-1.024	0.001
CD14 <sup>+</sup> CD16 <sup>++</sup> monocytes HL (cells/μl)	1.003	0.997-1.009	0.289
CD14 <sup>+</sup> CD16 <sup>++</sup> monocytes OL (cells/μl)	1.004	0.999-1.009	0.157

Cox Regression analyses includes monocyte subset counts, age, gender, prevalent CVD, eGFR, diabetes mellitus, CRP, HDL, LDL and mean blood pressure.

### ROC and Reclassification analyses for intermediate monocytes



### Expression analysis of surface proteins



## Conclusions

We confirmed intermediate monocytes to be independent predictors of cardiovascular outcome among CKD patients. Both suggested gating strategies led to similar results with the oblique approach providing a negligible improvement. Future studies into markers that allow for an unequivocal definition of intermediate monocytes may substantially improve the prediction power of these cells.