Gating Strategie zur Identifizierung von intermediären Monozyten bei Patienten mit chronischer Nierenerkrankung

Zawada AM[1,*], Fell LH[1,*], Untersteller K[1], Seiler S[1], Rogacev KS[1], Fliser D[1], Ziegler-Heitbrock L[2], Heine GH[1]

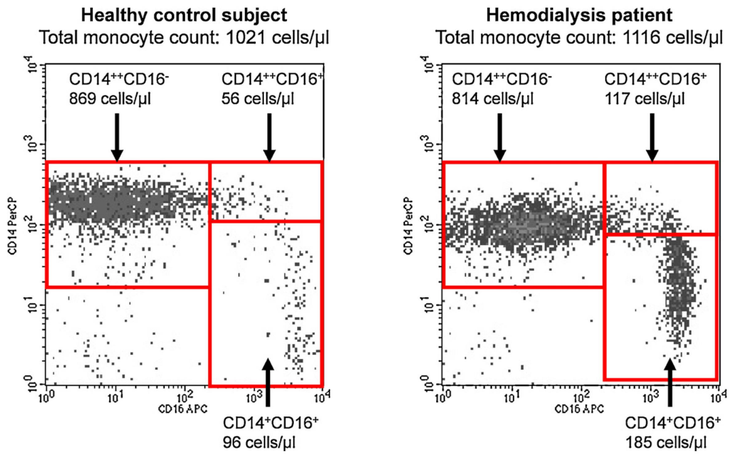
[1]Department of Internal Medicine IV, Saarland University Medical Center, Homburg, Germany
[2]Comprehensive Pneumology Center – EvA Study Center, Helmholtz Zentrum Muenchen – German Research Center

Saarland University Medical Center

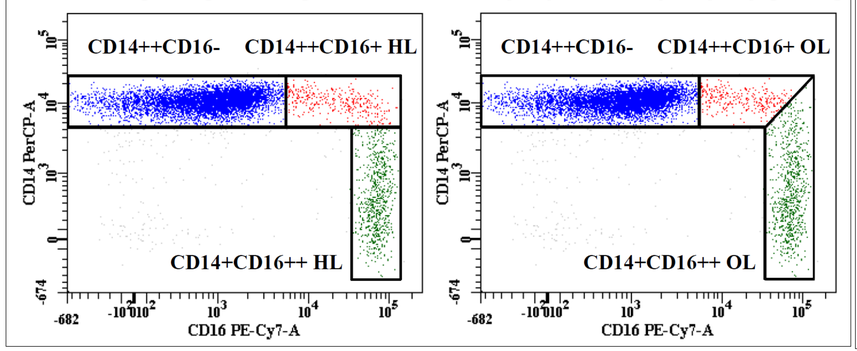
Introduction

Monocytes are a heterogeneous cell population consisting of three different subsets: classical CD14++CD16-, intermediate CD14++CD16+ and nonclassical CD14+CD16++ 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 delineation of intermediate from nonclassical monocytes: the "rectangular gating (RG) strategy" and the "trapezoid gating (TG) strategy" (see Figure). We compared the two gating strategies in a well-defined clinical cohort of patients with chronic kidney disease (CKD).

Monocyte subsets: healthy control *vs* patient with CKD



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



Results

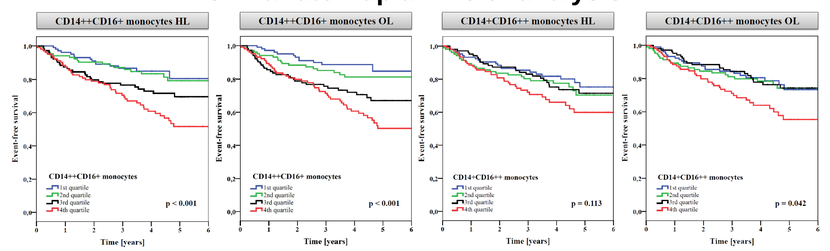
Baseline characteristics

	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/m²)	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/1.73m²)	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**

Cross-sectional analysis

	Intermediate monocytes					Nonclassical monocytes				
Variable	HL		OL		HS *vs* OL	HL		OL		HL *vs* OL
	r	p	r	p	p	r	p	r	p	p
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
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

Univariate Kaplan-Meier analysis



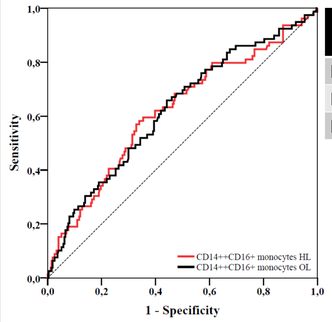
Results

Cox Regression analysis

	HR	CI	p
CD14++CD16+ monocytes HL (cells/µl)	**1.013**	**1.006-1.020**	**<0.001**
CD14++CD16+ monocytes OL (cells/µl)	**1.015**	**1.006-1.024**	**0.001**
CD14+CD16++ monocytes HL (cells/µl)	1.003	0.997-1.009	0.289
CD14+CD16++ 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.

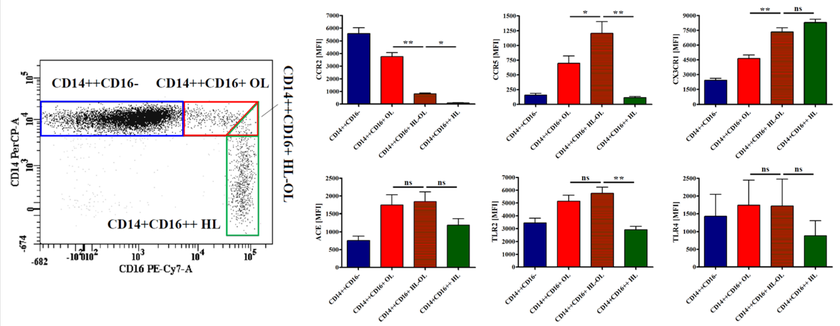
ROC and Reclassification analyses for intermediate monocytes



	No Event	Event	Total
Reclassification into higher quartiles	20	3	23
No Reclassification	227	74	301
Reclassification into lower quartiles	27	2	29

NRI_{events}:	1.3 %
$NRI_{non\text{-}events}$:	2.6 %
Net NRI:	**3.9 %**

Expression analysis of surface proteins



Conclusions

Intermediate monocytes were independent predictors of cardiovascular outcome irrespective of the applied gating strategy. Future studies should aim to identify markers that allow for an unequivocal definition of intermediate monocytes, which may further improve the prediction power for cardiovascular events of these cells.

Contact: Adam.Zawada@uks.eu