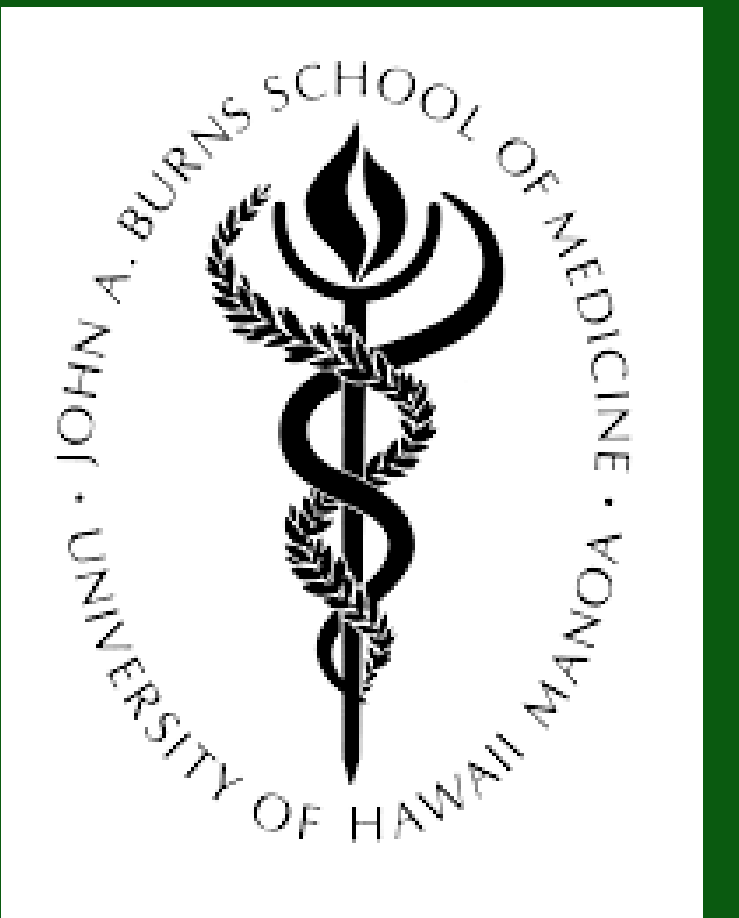




Elevated Numbers of Inflammatory Monocytes Predicts Increased Carotid Intimal Thickness in an HIV Uninfected Population

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Introduction

Atherosclerosis is an inflammatory disease characterized by the accumulation of atherosclerotic plaques and associated with the development of cardiovascular disease (CVD). Despite improvements in interventional therapies and treatments for atherosclerosis, CVD remains the leading cause of death in developed countries worldwide.

Based on their CD14 and CD16 surface expression, monocytes are classified into three subset populations: classical (CD14⁺⁺CD16⁻), intermediate or inflammatory (CD14⁺⁺CD16⁺), and non-classical or patrolling (CD14^{low}/CD16⁺⁺). Monocyte heterogeneity has been associated with the pathogenesis of atherosclerosis.

Reports on the role of intermediate monocyte subsets in the clinical progression of CVD are limited. The correlation between intermediate monocyte counts and carotid intima-media thickness (cIMT) in the general population has yet to be reported.

cIMT is a validated cardiovascular imaging test that measures carotid plaque accumulation using ultrasound. cIMT can predict cardiovascular events.

This study investigated the association between elevated intermediate monocyte counts and increased cIMT as predictors of CVD risk in the HIV-negative population.

Results

Baseline Characteristics of Study Participants

N	75
Age, years [median (Q1, Q3)]	54.46 (47.52, 60.29)
Male gender, n (%)	60 (80)
Ethnicity	
Caucasian, n (%)	50 (66.7)
African American, n (%)	1 (1.3)
Native Hawaiian/Pacific Islander, n (%)	0 (0)
Asian, n (%)	7 (9.3)
More than one race, n (%)	17 (22.7)
BMI, kg/m ² [median (Q1, Q3)]	26.84 (23.55, 29.40)
Hypertension, n (%)	21 (28)
Blood pressure	
Systolic Blood Pressure, mmHg [median (Q1, Q3)]	118 (109, 125)
Diastolic Blood Pressure, mmHg [median (Q1, Q3)]	69 (62, 76)
Fasting plasma glucose, mg/dL [median (Q1, Q3)]	78 (73, 85)
Diabetes mellitus, n (%)	3 (4)
LDL, mg/dL [median (Q1, Q3)]	114 (94, 136)
HDL, mg/dL [median (Q1, Q3)]	56 (46, 67)
Smoking history	
Current, n (%)	13 (17.3)
Past, n (%)	43 (57.3)
Never smoked, n (%)	19 (25.3)
10-year CHD risk estimated by FRS, % [median (Q1, Q3)]	4 (2, 7)

Pearson Correlation Between Monocytes and Predictors of CVD

Predictors of CVD	Total Monocytes *		Classical Monocytes *		Intermediate Monocytes *		Non-Classical Monocytes *	
	r	P-value	r	P-value	r	P-value	r	P-value
Age, years	0.18	0.119	0.16	0.181	0.28	0.016	0.26	0.024
BMI, kg/m ²	0.19	0.100	0.15	0.198	0.03	0.778	0.16	0.165
HDL, mg/dL	-0.06	0.580	-0.08	0.506	-0.02	0.842	-0.07	0.541
LDL, mg/dL	-0.03	0.800	0.01	0.941	-0.26	0.026	-0.19	0.099
Triglycerides, mg/dL *	-0.03	0.758	-0.11	0.320	-0.09	0.426	0.10	0.387
Total Cholesterol, mg/dL	-0.04	0.682	-0.04	0.689	-0.25	0.030	-0.15	0.185
HOMA-IR *	0.14	0.237	0.09	0.460	0.003	0.980	0.15	0.221
Framingham Risk Score *	0.35	0.003	0.35	0.003	0.32	0.006	0.17	0.144
CCA IMT	0.20	0.093	0.04	0.710	0.24	0.025	0.12	0.341
BIF IMT	0.10	0.413	0.01	0.930	0.22	0.031	0.22	0.050

Multiple Linear Regression Predicting Right Common Carotid Artery (RCCA) IMT* from Intermediate Monocytes

Parameter	Unstandardized Beta	Standardized Beta	P-Value
Intermediate Monocytes*	0.05	0.25	0.05
Age	0.002	0.23	0.05
Gender	0.01	0.06	0.62
BMI	0.002	0.16	0.22
LDL	4 x 10 ⁻⁴	0.19	0.11
HDL	-9 x 10 ⁻⁴	-0.24	0.06
Diabetes	0.008	0.03	0.79
Hypertension	-0.004	-0.03	0.81
Current Cigarette Smoker	-0.02	-0.11	0.31

Multiple Linear Regression Predicting Right Carotid Bifurcation (RBIF) IMT* from Intermediate Monocytes

Parameter	Unstandardized Beta	Standardized Beta	P-Value
Intermediate Monocytes*	0.06	0.25	0.05
Age	0.001	0.17	0.16
Gender	0.008	0.04	0.73
BMI	0.003	0.25	0.07
LDL	3 x 10 ⁻⁴	0.15	0.21
HDL	-2 x 10 ⁻⁴	-0.07	0.60
Diabetes	0.04	0.16	0.23
Hypertension	-4 x 10 ⁻³	-2 x 10 ⁻³	1.00
Current Cigarette Smoker	-0.009	-0.04	0.68

*Variable was log-10 transformed to correct for normality. A two-sided p-value ≤ 0.05 was considered statistically significant.

Discussion

- Higher intermediate monocyte count and older age were significantly associated with increased carotid intimal thickness at the common carotid region.
- Similarly, higher intermediate monocyte count was significantly associated with increased carotid intimal thickness at the bifurcation region.
- The correlation between intermediate monocyte count and cIMT at both the common carotid and bifurcation regions persisted even after adjusting for age, gender, BMI, LDL cholesterol, HDL cholesterol, diabetes mellitus, hypertension, and current cigarette smoking.
- These findings suggest a relationship between elevated intermediate monocyte subset counts and increased cIMT in the general population.

Limitations

- This study was an observational single time point assessment and had a limited sample size.
- This observational study could only demonstrate correlation, not causality.

Conclusions

- This study is the first to report the association between intermediate monocyte counts with cIMT at both the common carotid and bifurcation segments as predictors of CVD risk in the general population.
- The association between elevated intermediate monocyte counts and increased cIMT may contribute to developing novel therapeutic strategies in preventing cardiovascular events.
- Further investigation into the mechanism of monocytes in the development of atherosclerosis is warranted.

Acknowledgements

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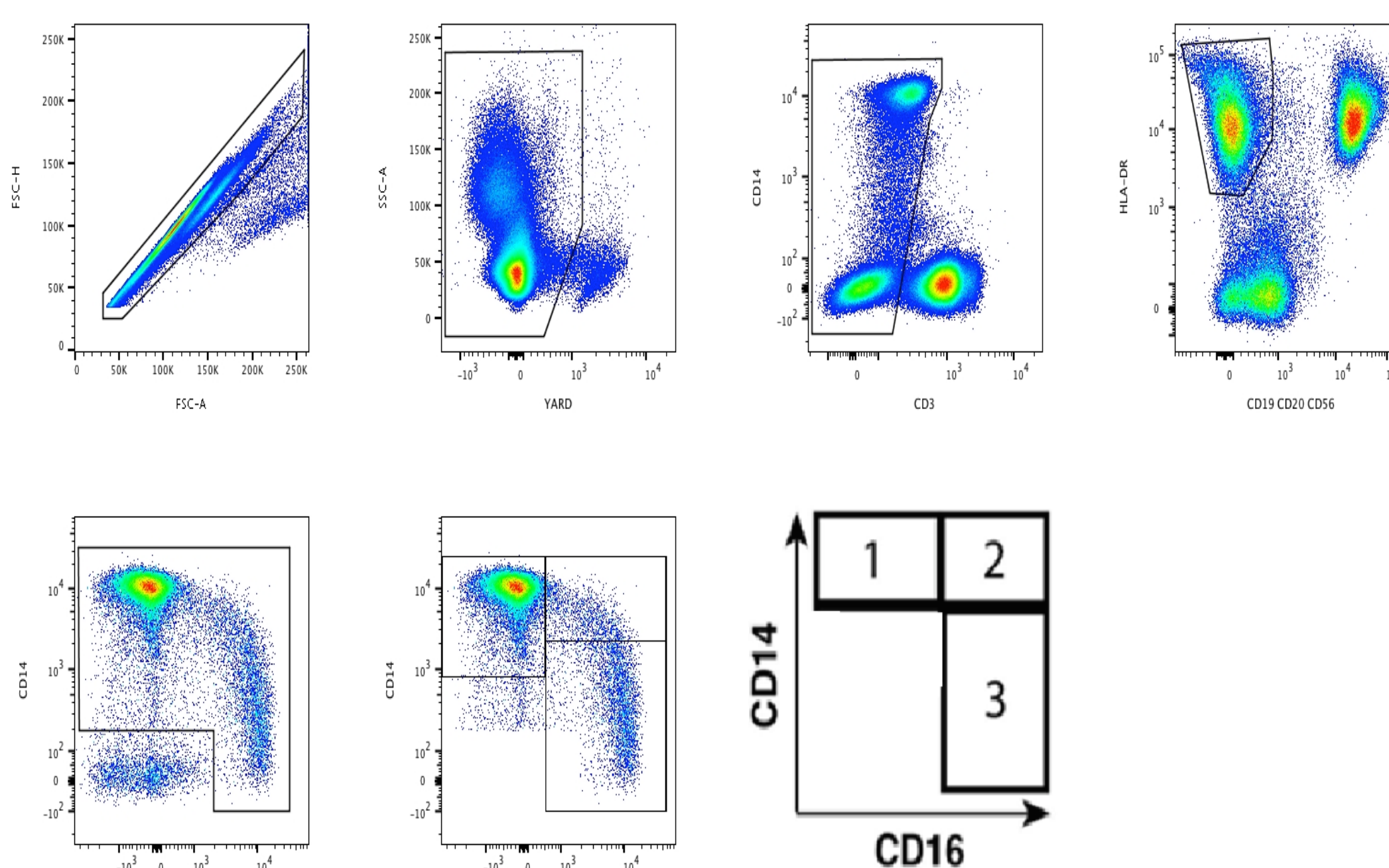
Materials and Methods

- This study is a cross-sectional analysis of HIV-negative subjects serving as the healthy comparison group in the Hawaii Aging with HIV-Cardiovascular (HAHC-CVD) study.
- HIV seronegative status was confirmed by enzyme-linked immunosorbent assay (ELISA).
- General medical history with special emphasis on CVD was obtained.
- The participants in this study did not have CVD.
- HAHC-CVD is a 5-year longitudinal cohort study that examined the role of oxidative stress and inflammation in HIV cardiovascular risk.

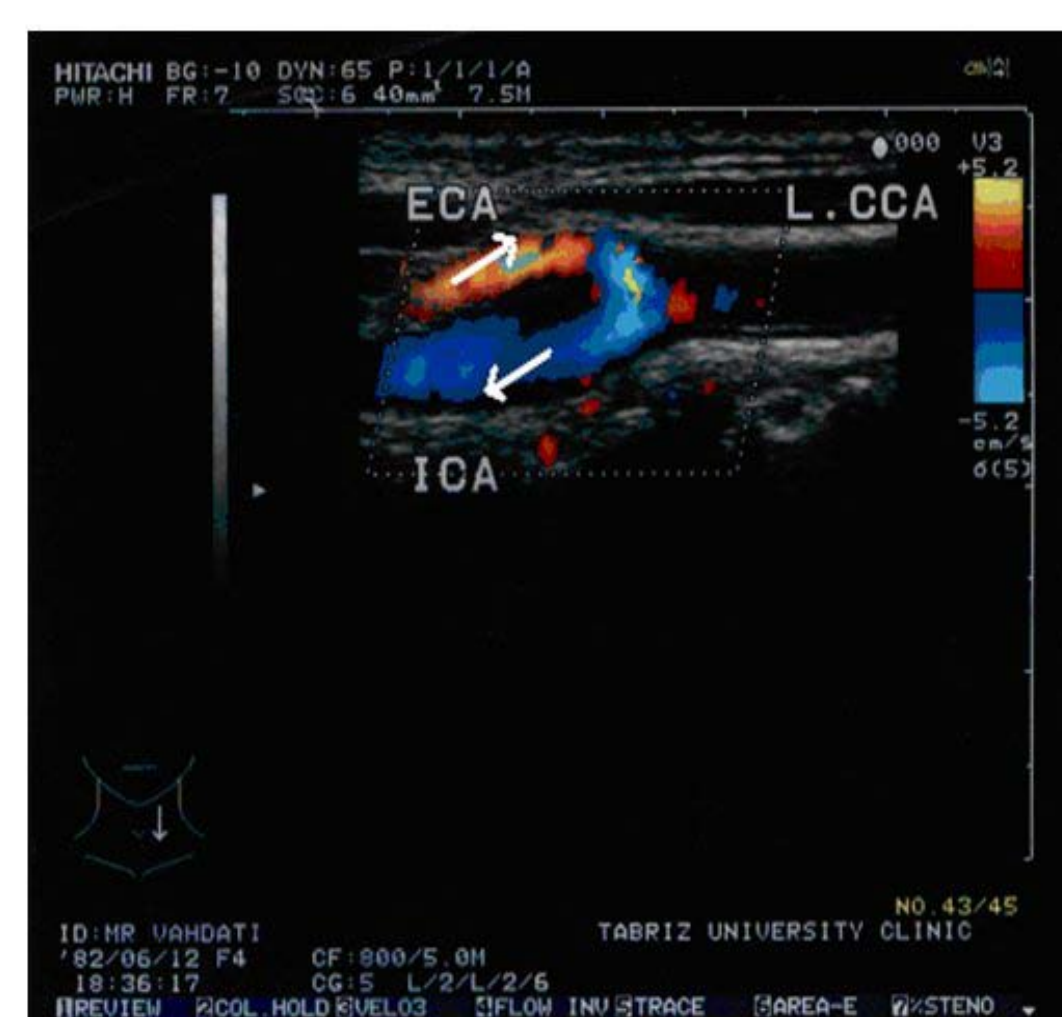
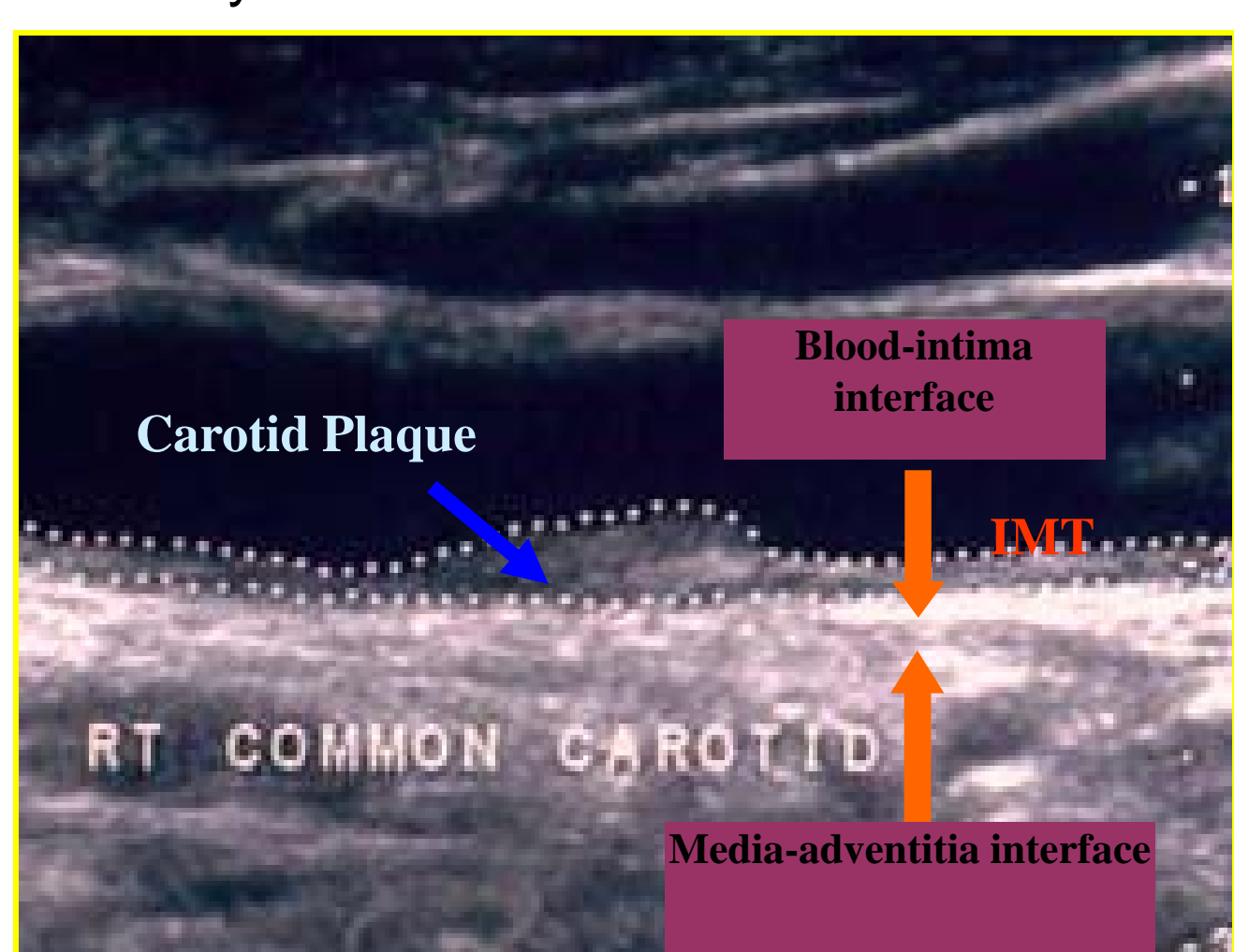
Carotid Intima-Media Thickness (cIMT) Measurements

- High-resolution B-mode ultrasound images of the right carotid artery was obtained.
- A single reader measured the intima-media thickness of the far wall of the distal carotid artery distal to the carotid artery bulb with automated edge detection (Images acquired at Queen's Medical Center in Honolulu, and analyzed at University of Southern California Atherosclerosis Research Unit).
- Measurements were obtained at each segment: common (CCA) and bifurcation regions (BIF).
- The CCA is located parallel to the skin surface, easily assessable for ultrasound imaging, and hence more reliable and reproducible.
- Atherosclerotic lesions appear later in the CCA than other measurement sites.
- The BIF region experiences low endothelial shear stress, which promotes the pathogenesis of atherosclerosis and may reflect early disease.

Cell Staining and Flow Cytometric Analysis



Multiparametric flow cytometry gating strategy to phenotype three distinct monocyte sub populations from peripheral blood based on CD16 and CD14 expression: (1) classical monocytes lacking CD16 expression (CD14⁺⁺CD16⁻) and those expressing CD16 comprised of (2) intermediate (CD14⁺⁺CD16⁺) and (3) non-classical (CD14^{low}/CD16⁺⁺) monocytes.



cIMT measures the distances between the blood-intima interface and media-adventitia interface. Measurements were taken from the common (CCA), internal (ICA) and external carotid artery (ECA). Outcome variables were based on CCA and BIF assessments.