Supplementary data

Supplementary Appendix 1. Study population (exclusion criteria)

Exclusion criteria were: (1) >40% angiographic stenosis in major vessels; (2) acute coronary syndrome presentation; (3) a history of myocardial infarction or cerebrovascular events within the past six months; (4) previous percutaneous coronary intervention or coronary artery bypass surgery; (5) use of radiographic contrast agents within 12 hours before catheterisation; (6) significant valvular heart disease; (7) advanced chronic kidney disease; (8) reduced left ventricular ejection fraction (<45%); (9) active malignancy; (10) local or systemic infectious disease within the past four weeks; (11) inflammatory diseases; (12) pregnant patients; and (13) those unable to provide written informed consent.

Supplementary Appendix 2. Statistical analysis

Continuous variables distributed normally were expressed as the mean±standard deviation, and those with a skewed distribution were expressed as the median (interquartile range). Categorical variables were expressed as frequency (percentage). For between-group comparisons, the unpaired t-test (or ANOVA) was used for normally distributed variables, Mann-Whitney U test (or Kruskal-Wallis rank-sum test) for non-normally distributed variables, and χ^2 test (or Fisher's exact test) for categorical variables. Univariate and multivariate (model 1: adjusted for age and sex; model 2: adjusted for age, sex, hypertension, dyslipidaemia, diabetes mellitus, body mass index, and estimated glomerular filtration rate) logistic regression analyses were performed to estimate the effects of CFR, HMR, and CMD on the risk of composite MACE. If more than one MACE were observed in a given patient, only the first event was included in the logistic regression analysis. CFR and HMR were included in the models either as continuous or as dichotomised variables. Patients were divided into two groups or four groups by predefined cut-off values of CFR and HMR for categorical analyses. The discriminatory power of the HMR for identifying composite MACE when adding HMR to CFR was evaluated by calculating net reclassification improvement and integrated discrimination improvement. For all tests, a two-

tailed p-value <0.05 was considered statistically significant. All statistical analyses were performed using JMP Pro software (SAS Institute, Inc., Cary, NC, USA) and R version 3.2.3 (R Foundation for Statistical Computing, Vienna, Austria).

Supplementary Appendix 3. The discriminatory power of HMR for composite MACE

We assessed the discriminatory power of HMR for composite MACE when adding HMR to CFR by calculating net reclassification improvement and integrated discrimination improvement. The discriminatory accuracy improved after adding HMR to CFR (net reclassification improvement 0.17, 95% CI: 0.02, 0.31; p=0.03; integrated discrimination improvement 0.01, 95% CI: 0.0001, 0.02; p=0.046) **(Supplementary Table 2)**.

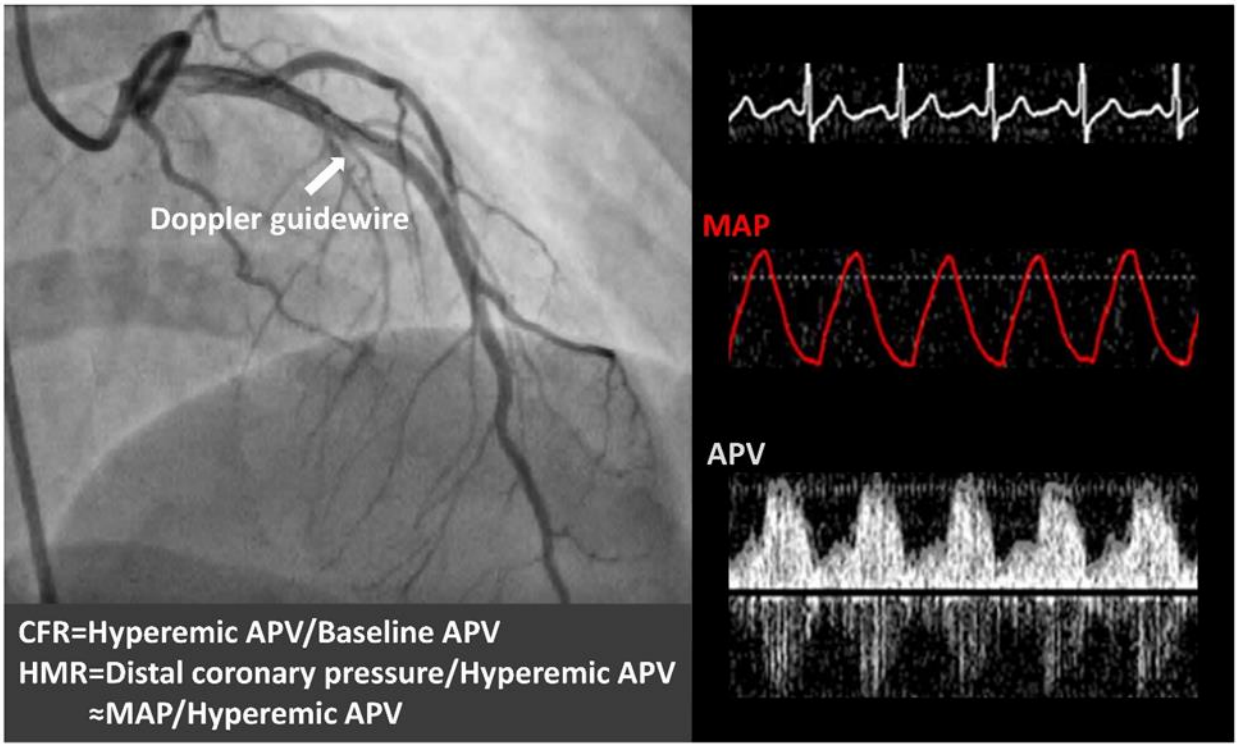
Supplementary Appendix 4. Logistic regression analysis to predict composite MACE

When we divided patients into four groups by CFR of ≤2.0, 2.01-2.5, 2.51-3.0, and >3.0 to assess the stepwise impact of CFR on composite MACE, there was a stepwise effect of CFR with CFR >3.0 significantly associated with a decreased risk of composite MACE compared to CFR ≤2.0 (OR 0.45, 95% CI: 0.24, 0.86; p=0.01; age- and sex-adjusted OR 0.40, 95% CI: 0.21, 0.78; p=0.01) **(Supplementary Figure 4A, Supplementary Figure 4B)**.

When we divided patients into four groups by HMR of ≤1.5, 1.51-2.0, 2.01-2.5, and >2.5 mmHg/cm/s based on the previous report using HMR of 2.5 mmHg/cm/s as a cut-off [23,24], HMR >2.5 was significantly associated with an increased risk of composite MACE compared to HMR ≤1.5 (OR 3.22, 95% CI: 1.55, 6.68; p=0.002; age- and sex-adjusted OR 2.79, 95% CI: 1.34, 5.83; p=0.01) **(Supplementary Figure 4C, Supplementary Figure 4D)**.

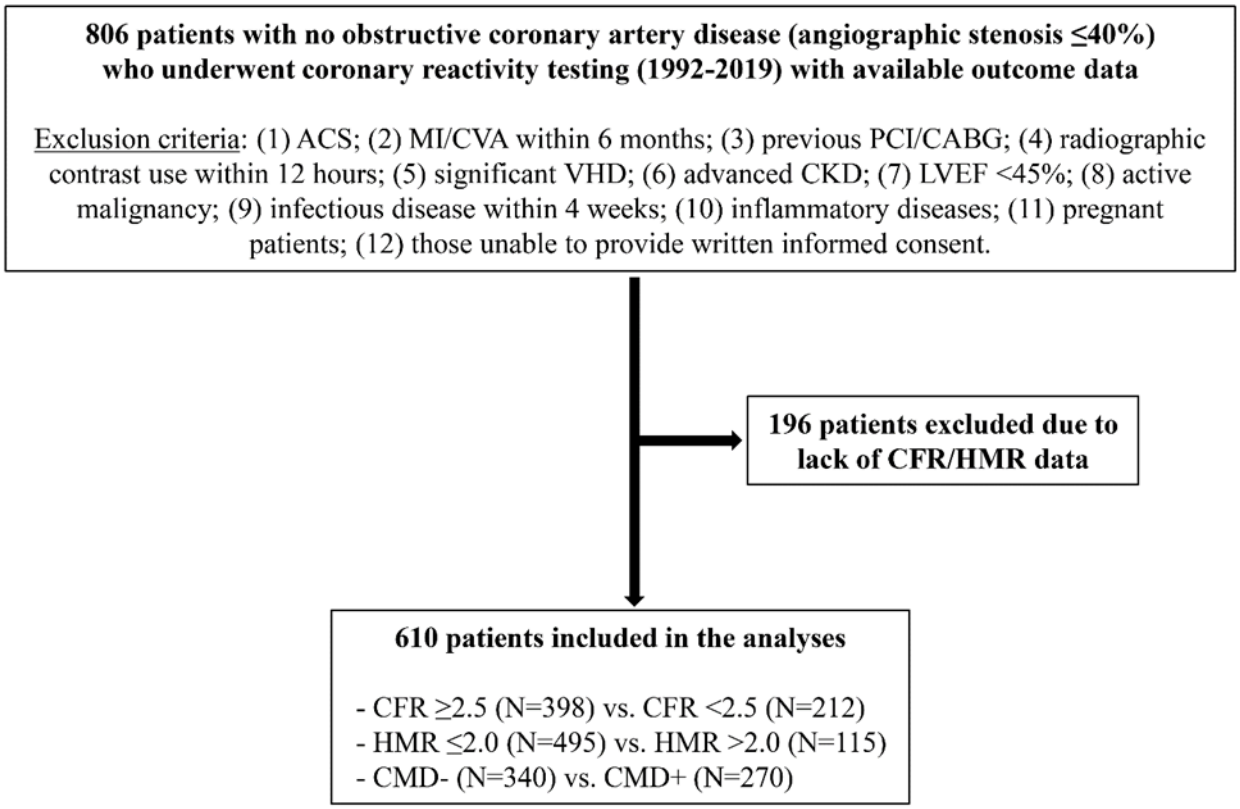
Supplementary Appendix 5. Limitations

First, because of its retrospective observational cohort design, causal associations cannot be derived from the current study. All the patients included in the present study were clinically referred for diagnostic coronary angiography, and coronary microvascular function testing was performed in patients without obstructive coronary artery disease. Selection bias cannot be avoided, thus affecting the generalisability of the findings. Second, clinical outcomes were collected by questionnaires. Therefore, recall bias might have affected the results; however, self-reported MACE were adjudicated and confirmed in patients whose medical charts were available by independent investigators (A. Ahmad and F. Sebaali) blinded to measurements. Since coronary reactivity testing was part of clinical assessment to guide therapy, patients and attending physicians were not blinded to the measurements, potentially affecting medical therapies with resultant change in outcomes. Dates of individual MACE events were not provided by patients, limiting our ability to perform time-dependent analysis. Also, our lack of data regarding the specific causes of death limits our ability to assess the association between CMD and the specific cause of death meaningfully. Interestingly, reduction of CFR is reported to be independently associated with cardiovascular as well as cancer mortality [40], which is consistent with our previous observation showing that abnormal peripheral microvascular vasomotor response is associated with increased risk of incident cardiovascular events and cancer [41-43]. Based on the fact that we did not observe a difference in rates in individual MACE except death between patients with and without CMD, CMD can be viewed as a marker of systemic microcirculatory health. Future studies are necessary to examine the relationship between CMD and specific causes of death. Furthermore, a cost-effectiveness study is required to warrant the application of CMD assessment for a broader range of patients; however, this study may postulate CMD as a potential marker to predict mortality and future MACE. The underlying mechanism linking CMD to future events needs further investigation. Third, we used aortic pressure during hyperaemia for the approximation of coronary pressure to calculate HMR. Given that only patients with non-obstructive coronary artery disease were included in the study, the difference between aortic pressure and coronary pressure is negligible and calculation of HMR using mean aortic pressure is valid.



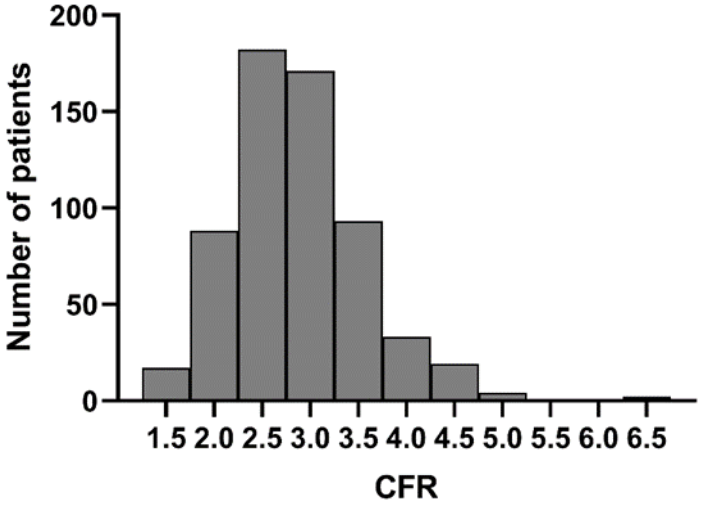
Supplementary Figure 1. Doppler flow measurement.

APV: average peak velocity; CFR: coronary flow reserve; HMR: hyperaemic microvascular resistance; MAP: mean arterial pressure

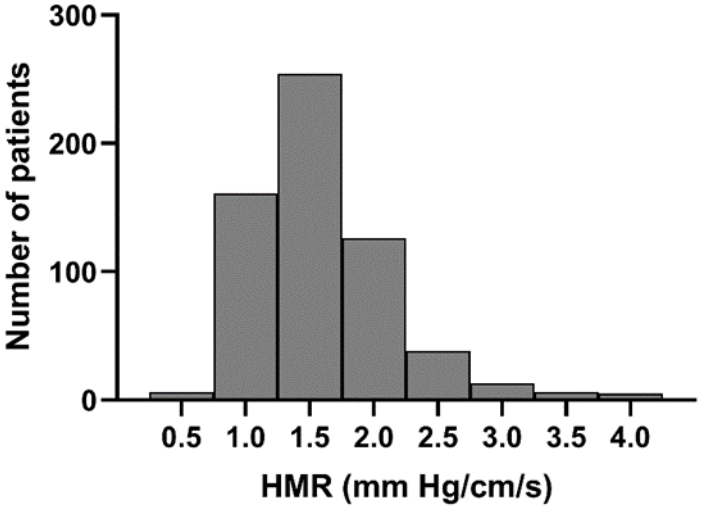


Supplementary Figure 2. Study flow chart.

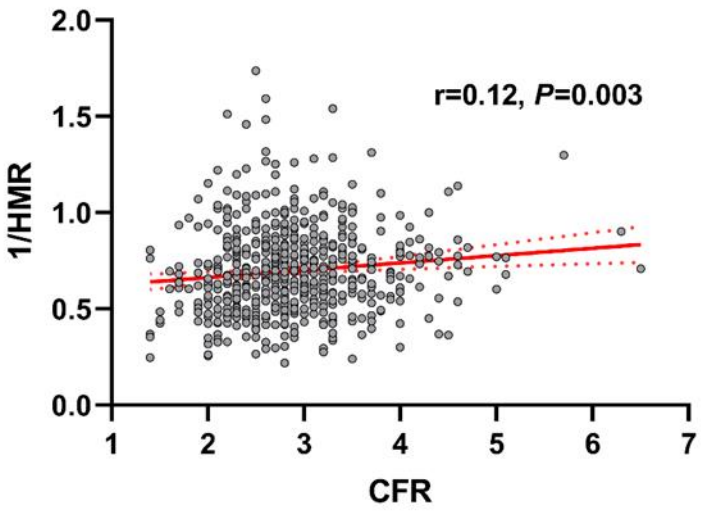
ACS: acute coronary syndrome; CABG: coronary artery bypass grafting; CFR: coronary flow reserve; CKD: chronic kidney disease; CMD: coronary microvascular dysfunction; CVA: cerebrovascular accident; HMR: hyperaemic microvascular resistance; LVEF: left ventricular ejection fraction; MAP: mean arterial pressure; MI: myocardial infarction; PCI: percutaneous coronary intervention; VHD: valvular heart disease



A) Distribution of CFR.

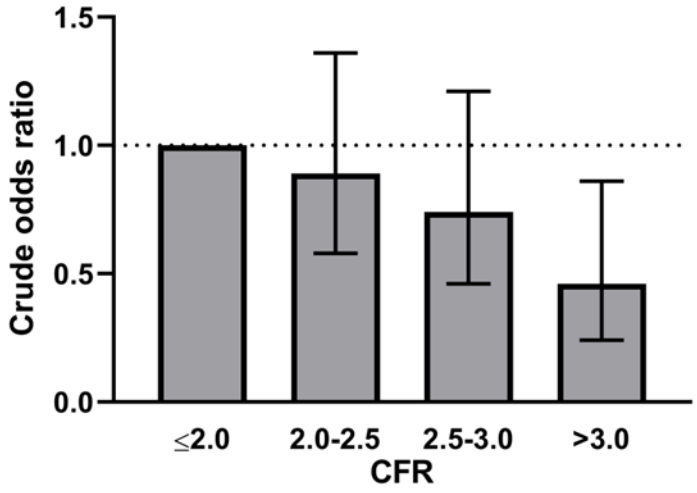


B) Distribution of HMR.

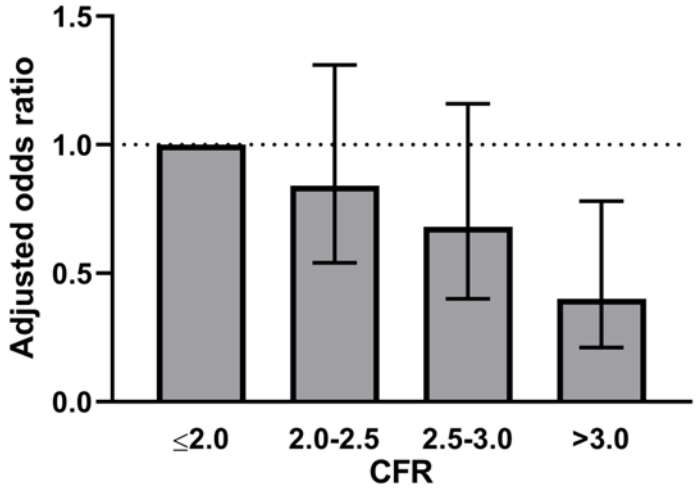


C) Correlation between CFR and HMR.

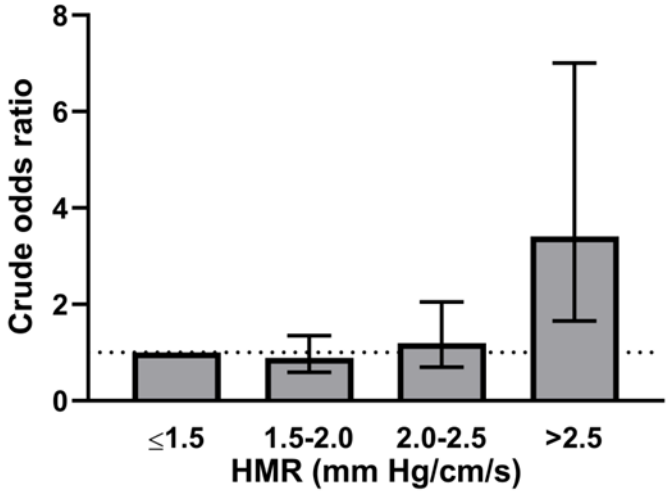
Supplementary Figure 3. Distribution of CFR (A) and HMR (B) and correlation between them (C).



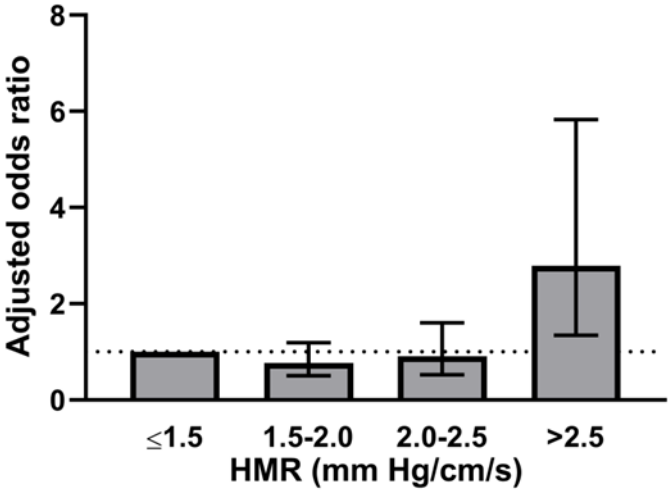
A) Univariate.



B) Multivariate (adjusted for age and sex).



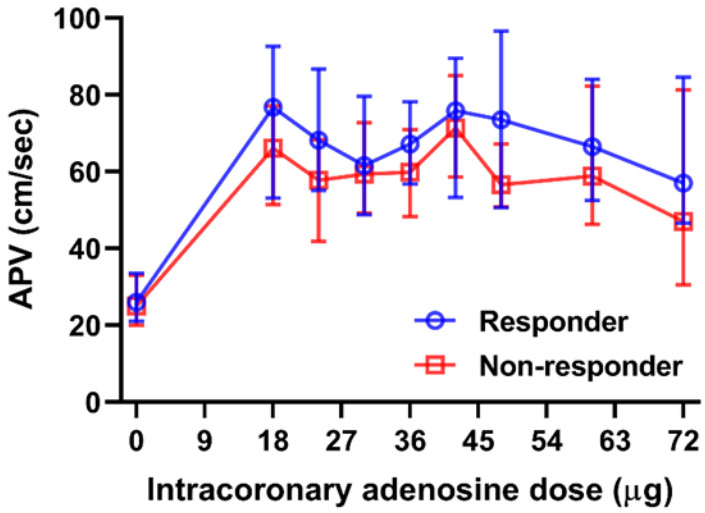
C) Univariate.



D) Multivariate (adjusted for age and sex).

Supplementary Figure 4. Stepwise risk assessment of MACE.

Bar graphs showing the odds ratio and 95% confidence interval for MACE. Patients were divided into four groups by CFR (≤2.0, 2.01-2.5, 2.51-3.0, and >3.0) or HMR (≤1.5, 1.51-2.0, 2.01-2.5, and >2.5 mmHg/cm/s). A) & C) Univariate. B) & D) Multivariate (adjusted for age and sex). CFR: coronary flow reserve; HMR: hyperaemic microvascular resistance; MACE: major adverse cardiovascular events



Supplementary Figure 5. Relationship between adenosine dose and APV.

Dose-response relationship between intracoronary adenosine and APV.

APV: average peak velocity

Supplementary Table 1. Baseline characteristics comparing patients with and without CMD.

	CMD− N=340	CMD+ N=270	*p*-value
Age, years	51.9±12.0	56.8±11.5	<0.0001
Male sex, n (%)	120 (35)	60 (22)	0.0004
Body mass index, kg/m^2	28.4 (24.7, 33.3)	27.5 (24.2, 32.1)	0.17
Smoking status, n (%)			
Never smoked	182 (54)	149 (55)	
Former smoker	127 (37)	102 (38)	0.64
Current smoker	31 (9)	19 (7)	
Diabetes, n (%)	38 (11)	32 (12)	0.79
Hypertension, n (%)	142 (42)	140 (52)	0.01
Dyslipidaemia, n (%)	195 (57)	167 (62)	0.26
Systolic BP, mmHg	125±17	128±20	0.03
Diastolic BP, mmHg	76±10	76±10	0.50
HbA1c, %	5.3 (5.1, 5.7)	5.4 (5.2, 5.7)	0.04
Total cholesterol, mg/dL	180 (155, 210)	187 (161, 220)	0.02
LDL-C, mg/dL	101 (75, 125)	100 (80, 132)	0.38
HDL-C, mg/dL	50 (42, 63)	54 (45, 68)	0.01
Triglyceride, mg/dL	107 (73, 173)	109 (74, 170)	0.85
eGFR, ml/min/1.73 m^2	77.1±16.9	74.1±17.8	0.03
CFR	3.1 (2.8, 3.5)	2.3 (2.1, 2.5)	<0.0001
HMR, mmHg/cm/sec	1.35 (1.16, 1.59)	1.83 (1.38, 2.22)	<0.0001

BP: blood pressure; CFR: coronary flow reserve; eGFR: estimated glomerular filtration rate; HbA1c: haemoglobin A1c; HDL-C: high-density lipoprotein cholesterol; HMR: hyperaemic microvascular resistance; LDL-C: low-density lipoprotein cholesterol

Supplementary Table 2. Discriminatory power of HMR for composite MACE.

	CFR	CFR+HMR	*p*-value
C-statistics	0.56	0.59	0.16
Net reclassification index	0.17, 95% CI [0.02, 0.31]		0.03
Integrated discrimination improvement	0.01, 95% CI [0.0001, 0.02]		0.046

CFR: coronary flow reserve; HMR: hyperaemic microvascular resistance