Supplementary Table 1. Examples of blood-based assays in clinical studies of CV diseases

Circulating biomarker	Type of study	Study size	Technique	Results	Clinical use	Ref.
CECs	Case-control	24 HT, 25 DN, 27 HFpEF, 25 HFrEF compared to 11 controls.	Flow cytometry	Increased CEC number in DN and HFpEF patients.	CEC counts is a putative diagnostic biomarker for detection of DN and HFpEF.	[20]
CECs/ET-1	Case-control	15 cases of left-to-right shunt CHD without PAH, 26 cases of CHD complicated with mild PAH, and 17 cases of CHD complicated with moderate-severe PAH compared to 30 controls	Flow cytometry ELISA	Higher levels of CECs and ET-1 in the group of moderate-severe PAH compared to other study groups. Mean pulmonary artery pressure positively correlated with percentage of CECs as well as ET-1 production.	CECs and ET-1 could be used as clinical biomarkers to define management of PAH patients.	[42]
EPCs/oxLDL	Cross-sectional, observational	33 patients with stable CHD	Flow cytometry ELISA	Patients with stable CHD had a high prevalence of coronary endothelial dysfunction, which was associated with lower numbers of circulating EPCs. A positive correlation between oxLDL and EPCs suggest a statin-mediated host-repair mechanism.	Combination of EPCs, oxLDL, and QCA to promote endothelial function which, in turn, may improve cardiovascular health.	[43]
cfDNA	Case-control	54 acute ischemic stroke patients treated with intravenous thrombolysis compared to 15 controls	RT-PCR	Lower cfDNA levels was found in patients who neurologically improved at 48 h	cfDNA could be a surrogate marker for monitoring tPA efficacy by the prediction of short-term neurological outcome.	[21]
cfDNA	Prospective	160 AMI patients compared with 30 controls	Quantitation by using fluorescence detection	cfDNA levels were higher AMI patients compared with controls and discriminated severity of the disease.	Circulating cfDNA levels in AMI patients may be an alternative approach to monitor the disease and identify high-risk individuals that may undergo reinfarction or HF.	[47]
dd-cfDNA	Prospective cohort study	21 pediatric and 44 adult patients undergoing HTx (565 plasma samples)	Quantitative-GTD based on shotgun SNP genotyping	Higher levels of dd-cfDNA provided an early diagnosis of acute rejection.	GTD may detect acute reactions up to 5 months before EMB suggesting a potential to complement or replace existing gold-standard approaches.	[22]
circANRIL	Cross-sectional cohort study	Endarterectomy samples from 218 CHD patients	Proteomic screening, bioinformatics	*circANRIL* can regulate pre-rRNA maturation	*circANRIL* may confer atheroprotection by modulating apoptosis	[49]

		undergoing vascular surgery	and functional studies.	controlling ribosome biogenesis and nucleolar stress.	and proliferation in human vascular cells and tissues. (Therapeutic agent ?)	
circRNAs	Case-control	12 CHD patients and 12 controls	RNA microarray	hsa_circ_0124644 was significantly upregulated in CHD patients respect with controls.	hsa_circ_0124644 can be used as a diagnostic biomarker of CHD.	[50]
MICRA	Case-control	472 patients with AMI at the time of reperfusion after percutaneous intervention	qRT-PCR	MICRA levels were lower in patients with reduced EF compared to mid-range EF or preserved EF.	MICRA may be a useful predictive biomarker of post-IMI LV dysfunction.	[51]
hsa_circ_0037911	Case-control	100 EH respect with 100 controls	qRT-PCR	hsa_circ_0037911 levels were significantly higher in EH patients than controls and positively correlated with Scr.	hsa_circ_0037911 may regulate the concentration of Scr providing a stable biomarker for early diagnosis of EH.	[52]
cfDNA	Case-control	57 STEMI patients respect with 83 controls	ddPCR	Higher levels of cardiac cfDNA was observed STEMI patients respect with controls.	Measurements of cardiac cfDNA capture cardiomyocyte cell death associated with myocardial infarction, and that the cardiac cfDNA assay can identify myocardial cell death early after ischemia ensues.	[55]
miR-92a	Prospective	40 ACS patients with prior history of CHD and T2D, 40 ACS patients with diagnosis of CHD for more than 2 years with no history of T2D, 68 controls	qRT-PCR	Higher levels of miR-92a was associated with an increased risk of ACS in CHD-T2D group.	A multipanel of miR-92a, HbA1c, and SBP may have a powerful predictive value of ACS in T2D	[63]
miRNAs	Prospective	137 patients with AHF, 20 with CHF, 8 with acute exacerbation of COPD, and 41 controls	qRT-PCR	Lower levels of miR-18a-5p, miR-26b-5p, miR-27a-3p, miR-30e-5p, miR-106a-5p, miR-199a-3p, and miR-652-3p were associated with AHF group. Lower levels of let-7i, miR-18b, miR-18a, miR-223, miR-301a, miR-652 and miR-423 may be predictive for mortality in AHF patients	These miRNA panels may be useful to predict increasing acuity in and mortality in AHF patients. Moreover, these molecules may suggest novel miRNA-based therapies.	[65]
miR-132	Case-control	A subset of 953 patients CHF from the GISSI-HF trial	qRT-PCR	Higher levels of miR-132 were independently associated with	miR-132 may be a useful risk biomarker of risk of future hospitalization for HF	[66]

		and controls		younger age, better renal filtration, ischemic atiology and severity of HF symptoms, higher DBP, higher cholesterol, and male sex.	patients	
miRNAs	Prospective	834 CHF patients from cohort 1 and 1369 CHF patients from cohort 2 and controls	qRT-PCR	Higher levels of miR-1254 and miR-1306-5p were significantly associated with all-cause mortality and risk hospitalization in both cohorts.	miR-1254 and miR-1306-5p may be useful prognostic biomarkers in CHF.	[67]
NETs	Prospective	48 patients after cardiac surgery with cardiopulmonary bypass and controls	ELISA	Circulating histone levels were higher in patients with adverse events postoperatively.	Circulating histones may be used as a prognostic indicator for patients after cardiopulmonary bypass.	[69]
NETs	Prospective	87 patients undergoing cardiac surgery and controls	ELISA	Higher serum levels of ds-DNA were observed postoperatively and associated with perioperative renal dysfunction.	dsDNA levels may be predictive of NET amount in serum suggesting a poor prognostic indicator for patients after cardiopulmonary bypass	[70]
EVs containg miRNAs (microvesicles)	Prospective	176 patients with stable CHD and controls	qRT-PCR	Higher levels of EVs carrying miR-126 and miR-199a were associated with a lower risk of future MACEs.	EV-related miR-126 and miR-199a may be predictive of better prognosis in CHD patients.	[80]
EVs containg miRNAs (exosomes)	Case-controls	145 AMI patients and controls	qRT-PCR	Hypoxia-induced miR-30a was highly enriched in exosomes from the serum of AMI patients respect with controls.	Exosome-related miR-30a may be a useful indicator of autophagy of cardiomyocytes. (Novel therapeutic target?)	[81]
EVs containg miRNAs (exosomes)	Prospective	21 patients developing HF within 1 year after AMI and 65 controls	qRT-PCR	Higher levels of exosome-related miR-192, miR-194, and miR-34a were associated with HF development *via* the p53 pathway.	Exosome-related miR-192, miR-194, and miR-34a may be useful prognostic predictors of ischemic HF development after AMI.	[82]
Metabolites	Prospective	7256 subjects from the National Finnish FINRISK study	NMR	Changes in expression levels of phenylalanine, MUFA, omega-6 fatty acids, and DHA were associated with cardiovascular events in a 15 year follow-up.	MUFA, omega-6 fatty acids, and DHA may be useful additional prognostic biomarkers of cardiovascular events.	[88]
Metabolites	Prospective	1,670 individuals from three independent	MS-HPLC	Changes in plasma levels of lipid fractions,	Lipid fractions, glucose, valine, ornithine, glutamate,	[89]

		cohorts of study from the Swedish Twin Register		glucose, valine, ornithine, glutamate, creatinine, glycoproteins, citrate and 1,5-anhydrosorbitol were associated with CHD onset.	creatinine, glycoproteins, citrate and 1,5-anhydrosorbitol may be useful predictive biomarkers for CHD onset in the general population.	
Metabolites	Prospective	3924 subjects from three independent cohorts without HF	MS	Changes in circulating levels of the haem breakdown product urobilin and sphingomyelin (30:1) were associated with development of HF.	Changes in circulating levels of the haem breakdown product urobilin and sphingomyelin (30:1) may be useful non-invasive predictors of HF in the general population.	[90]

Abbreviations: ACS: acute coronary syndrome; AHF: acute heart failure; AMI: acute myocardial infarction; CEC: circulating endothelial cells; CHD: coronary heart disease; CHF: chronic heart failure; cfDNA: cell-free DNA; circRNAs : circular RNAs; circANRIL: circular antisense non-coding RNA in the INK4 locus; COPD: chronic obstructive pulmonary disease; DBP: diastolic blood pressure; dd-PCR: digital droplet polymerase chain reaction; dd-cfDNA: donor derived cell-free DNA; DHA: docosahexaenoic acid; DN: diabetic nephropathy; dsDNA: double strand DNA; EH: essential hypertension; ELISA: enzyme-linked immunosorbent assay; EPCs: endothelial progenitor cells; ET-1: endothelin 1; EV: extra-cellular vesicles; HbA1c: glycosylated hemoglobin; FAM101A: refilin A; GTD: genome transplant dynamics; HFpEF: heart failure preserved ejection fraction; HFrEF: heart failure reduced ejection fraction; HPLC: high performance liquid chromatography; HT: hypertension; HTx: heart transplantation; LV: left ventricle; MACEs: major adverse cardiovascular events; miRNAs: micro-RNAs, MS: mass spectrometry; MUFA: monounsaturated fatty acids; NETs: neutrophil extracellular traps: NMR: nuclear magnetic resonance; NSTE-ACS: non-ST-segment elevation acute coronary syndrome; oxLDL: oxidized low-density lipoprotein; pre-rRNA: precursor ribosomal RNA; QCA: quantitative coronary angiography; qRT-PCR: quantitative real-time polymerase chain reaction; SBP: systolic blood pressure; Scr: serum creatinine: SNP: single nucleotide polymorphisms; STEMI: acute ST-elevation myocardial infarctions; T2D: type 2 diabetes; tPA: tissue plasminogen activator.