Cell-free DNA as a potential biomarker for acute myocardial infarction: A systematic review and meta-analysis

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ABSTRACT

Background: Tissue necrosis releases cell-free deoxyribonucleic acid (cfDNA), leading to rapid increases in plasma concentration with clearance independent of kidney function.
Aim: To explore the diagnostic role of cfDNA in acute myocardial infarction (AMI).
Methods: This systematic review and *meta*-analysis included studies of cfDNA in patients with AMI and a comparator group without AMI. The quality assessment of diagnostic accuracy studies-2 (QUADAS-2) tool was used, with AMI determined from the criteria of the original study. Standardised mean differences (SMD) were obtained using a random-effects inverse variance model. Heterogeneity was reported as I^2. Pooled sensitivity and specificity were computed using a bivariate model. The area under the curve (AUC) was estimated from a hierarchical summary receiver operating characteristics curve.
Results: Seventeen studies were identified involving 1804 patients (n = 819 in the AMI group, n = 985 in the comparator group). Circulating cfDNA concentrations were greater in the AMI group (SMD 3.47 (95%CI: 2.54–4.41, p < 0.001)). The studies were of variable methodological quality with substantial heterogeneity (I^2 = 98%, p < 0.001), possibly due to the differences in cfDNA quantification methodologies (Chi^2 25.16, p < 0.001, I^2 = 92%). Diagnostic accuracy was determined using six studies (n = 804), which yielded a sensitivity of 87% (95%CI: 72%-95%) and specificity of 96% (95%CI: 92%-98%). The AUC was 0.96 (95%CI: 0.93–0.98). Two studies reported a relationship between peak cfDNA and peak troponin. No studies reported data for patients with pre-existing kidney impairment.
Conclusion: Plasma cfDNA appears to be a reliable biomarker of myocardial injury. Inferences from existing results are limited owing to methodology heterogeneity.

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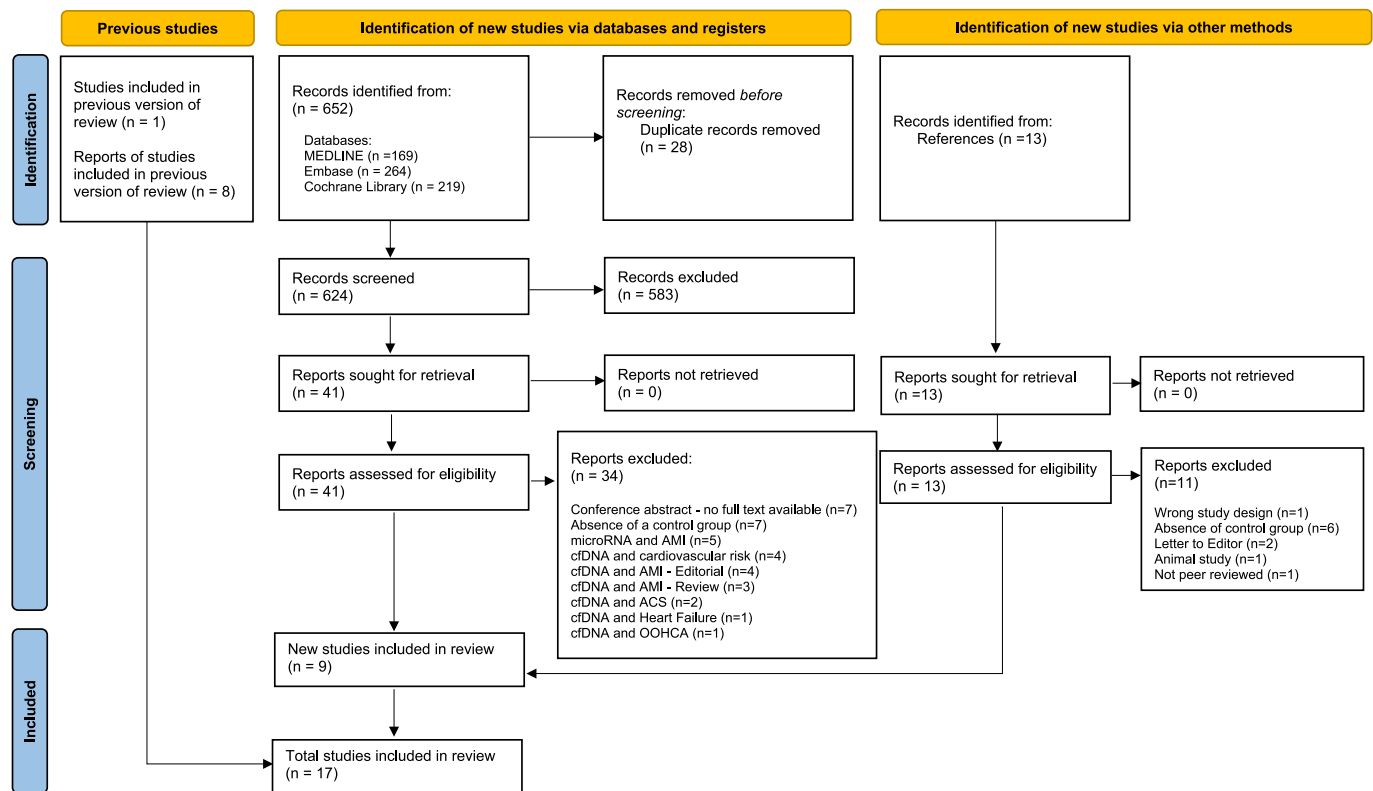*E-mail address:* tanyilin@gmail.com (E. Tan).

Fig. 1. PRISMA 2020 flow diagram for updated systematic reviews.

1. Introduction

Cell-free DNA (cfDNA) was first described by Mandel and Metais in 1948 [1]. Its origin is probably linked to three phenomena: cellular apoptosis, necrosis and, to a lesser extent, extracellular vesicle active secretion [2]. Healthy individuals have low plasma concentrations of cfDNA because of rapid elimination mainly by macrophages in the liver and spleen [3].

Cardiac troponin is established as the 'gold standard' for the diagnosis of acute myocardial infarction (AMI). However, this biomarker has three main limitations. Firstly, the major limitation of standard cardiac troponin assays (cTn) (cardiac troponin I (cTnI) and cardiac troponin T (cTnT)) is a delay to increased plasma concentration of 4–10 h after the onset of chest pain [4]. This has led to the development of high-sensitivity cardiac troponin (hs-cTn) assays (high-sensitivity cardiac troponin T (hs-cTnI) and high-sensitivity cardiac troponin I (hs-cTnI)), with plasma concentrations detected within three hours of symptoms onset [5]. Secondly, cardiac troponin concentration are challenging to interpret in patients with chronic kidney disease (CKD), a common co-morbidity in patients with AMI [6]. Lastly, there is an extended window during which troponin concentrations are increased, making it challenging to determine ongoing ischaemia in patients with an AMI.

AMI is caused by prolonged ischaemia time, rapidly depleting cellular adenosine triphosphate (ATP), triggering an ischemic cascade and eventual induction of cell death through apoptosis or necrosis [7]. The disruption of the membrane of necrotic cardiomyocytes causes DNA fragments to leak out and circulate in the peripheral circulation. Circulating cfDNA is detected within two hours from the onset of chest pain [8], and is not increased in patients with kidney dysfunction [9,10].

This systematic review aimed to clarify the potential role of cfDNA as an early biomarker in diagnosing patients with AMI. The primary hypothesis was that cfDNA concentration is greater in those patients presenting with AMI compared to a non-AMI comparator group.

2. Methods

This review was conducted according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA)–Diagnostic Test Accuracy (DTA) statements [11,12] (Table S1 and S2). Details of the protocol were registered prospectively with PROSPERO (CRD42022333190).

2.1. Data source and study search

A comprehensive search strategy was conducted using three databases (MEDLINE, Embase and the Cochrane Library) from inception to 17 May 2022. The medical subject heading terms and keywords included a broad range of descriptors for "acute myocardial infarction" and "cfDNA". Additional articles were identified by manually reviewing the references of the included articles. No language restrictions were applied. Further details are available in Table S3.

2.2. Eligibility criteria - study selection

The inclusion criteria were: (1) studies that reported cfDNA concentrations at admission in patients with AMI and (2) a comparator group. Exclusion criteria were: (1) reviews, letters, case series and case reports: and (2) studies that did not contain a comparator group. Two reviewers (ET and JZ) independently screened titles and abstracts and consecutively assessed the full texts of potentially relevant articles. Any reviewer disagreement was resolved by consensus.

2.3. Data extraction

The following study data were extracted in duplicate by two authors (ET and JZ): first author, publication year, country and region, hospital, funding source, study methodology, age, gender, sample size, time from admission to when blood samples were collected, processing time, DNA

Table 1
Study characteristics of the included studies.

Author	Year	Country	Study type	Number of patients	Age, years mean (SD)	Male, n (%)	Time of blood taken	Reference tests for the diagnosis of AMI
Agiannitopoulos	2020	Greece	Case-control	80 AMI 50 controls	62.1 (11.0) 59.3 (9.8)	NR	NR	STEMI defined based on the Third Universal definition of Myocardial Infarction
Antonatos	2006	Greece	Case-control	13 AMI 30 controls	NR NR	7 (54%) NR	On admission and for 5 consecutive days	AMI patients who underwent thrombolysis within six hours of chest pain
Arafat	2017	Egypt	Case-control	50 AMI 30 controls	57.7 (9.6) 54.6 (10.1)	40 (80%) 22 (73%)	On admission and Day 3	AMI defined based on the joint ESC/ACC criteria
Chang	2003	China	Case-control	55 AMI 274 controls	NR NR	NR NR	NR	Did not define
Cui	2013	China	Case-control	49 AMI 60 controls	NR 56.7 (11.1)	NR 34 (56.7%)	Within 6 h after the onset of chest pain	STEMI defined based on the ACC/AHA 2007 guidelines
Destouni	2009	Greece	Case-control	47 AMI 100 controls	61.7 (11.8) 65.4 (6.5)	34 (72%) 44 (44%)	On admission, Day 1, Day 2, Day 3, Day 4 and Day 5	AMI defined based on the joint ESC/ACC 2000 consensus guidelines
Jing	2011	China	Case-control	22 AMI 60 controls	NR 18–56	NR 45 (75%)	Within 6 h of admission after onset of chest pain. 5 random patients had blood samples collected on admission, and Day 1, Day 2 and Day 3 after PCI.	AMI defined according to the clinical symptoms, 12-lead ECG and positive cardiac biomarkers (cTnI, CK-MB, MYO)
Lou	2015	China	Case-control	120 AMI 60 controls	40–80 40–80	60 (50%) 30 (50%)	Within 6 h of admission after onset of chest pain	AMI defined according to clinical symptoms, 12-lead ECG and positive cardiac biomarkers (cTnI, CK-MB, MYO, LDH)
Qin	2016	China	Cohort study	38 AMI 32 controls	54.8 (14.3) 59.3 (12.7)	31 (82%) 25 (78%)	On admission, 12 h post PCI, 24 h post PCI, 48 h post PCI	AMI defined based on the joint ESC and ACC/AHA guidelines
Rainer	2006	China	Case-control	10 AMI 21 controls	68 (13) 64 (8)	NR 8 (38%)	On admission and 6 h later, then followed up for 2 years	STEMI - AMI defined based on the joint ESC/ACC 2000 consensus definition
Ren	2022	China	Observational	20 AMI (cohort 1) 20 AMI (cohort 2) 116 AMI (cohort 3) 25 controls	NR	NR	Cohort 1: after PCI Cohort 2: On admission, Day 1 post PCI, Day 2 post PCI Cohort 3: within 24 h of symptoms onset	AMI defined based on Fourth Universal definition of Myocardial infarction
Shimony	2010	Israel	Observational	16 AMI 47 controls	50.3 (12.5) 26.3 (4.7)	14 (93%) 25 (53%)	On admission, and three additional time points, 5–8 h apart	STEMI – Did not define
Veiko	2007	Russia	Observational	7 AMI 18 controls	78 (7) 38 (16)	4 (57%) 11 (61%)	Within 3 h after the development of AMI	Did not define
Wang	2015	China	Case-control	25 AMI 25 controls	59.3 (13.4) 64.5 (12)	21 (84%) 18 (72%)	Within 8 h of admission and Day 2 post PCI	AMI defined based on the joint ESC/ and ACC/AHA guidelines
Xie	2018	China	Case-control	100 AMI 30 controls	59 (10) 58 (5)	71 (71%) 15 (50%)	Onset of AMI, 5 consecutive days and monthly intervals for 5 months	AMI defined based on the joint ESC/ACC 2000 consensus guidelines
Xu	2012	China	Case-control	40 AMI 40 controls	44–66 40–60	28 (70%) 22 (55%)	Within 48 h after onset of chest pain and approximately 8 weeks post treatment	AMI defined based on the Guidelines for the Diagnosis and Treatment of Acute Myocardial Infarction, revised by the Cardiovascular Branch of the Chinese Medical Association
Zemmour	2018	Israel	Observational	31 AMI 83 controls	NR 35.3	NR 37 (45%)	Within 13 h of onset of chest pain and 6 h intervals up to 60hr from hospital admission	AMI diagnosed with STE > 1 mm in two or more contiguous leads. Final diagnosis was determined after coronary angiography with evidence of coronary obstruction or thrombus.

ACC: American College of Cardiology, AHA: American Heart Association, ESC: European Society of Cardiology, NR: Not Reported.

(a)

(b)

Fig. 2. Methodological quality of the included studies (a) individual assessment (b) Summary of methodological quality of included studies.

poor quality [15] Table S5.

2.5. Statistical analyses

For studies that used more than one cfDNA quantification method, data from the method that provided the highest area under the Receiver Operative Characteristic (ROC) curve value was used. If the original data were reported as medians and interquartile range or medians and 95% confidence interval (CI) or means and standard error, they were transformed into means and standard deviations. Differences in cfDNA concentrations between the AMI and the comparator groups were quantified as Hedges' g standardised mean differences (SMD), with 95% CI combined using a random-effects inverse variance model, and findings graphically represented using forest plots [16]. Heterogeneity was tested using the Chi^2 and I^2 statistic, with heterogeneity defined as Chi^2 $p < 0.10$ or $I^2 > 50\%$. Sources of heterogeneity were explored using subgroup analysis stratified according to (1) the type of quantification method used (optical technique, quantitative polymerase chain reaction (qPCR), DNA methylation analysis), (2) whether AMI was defined according to the European Society of Cardiology (ESC) or American Heart Association (AHA) or American College of Cardiology (ACC) consensus guidelines (yes, no) and (3) whether there was funding support (yes, no).

To determine diagnostic accuracy, an AMI was determined from the criteria of the original study. Estimated pooled sensitivities, specificities, and AUCs were obtained using bivariate modelling [17] and graphically depicted using the summary ROC curve. Sensitivity analysis was performed for studies that measured cardiomyocyte-specific cfDNA and studies that did not define AMI according to the ESC or AHA or ACC consensus guidelines. Publication bias was assessed through visual inspection of funnel plots and Egger's regression test. Statistical analyses were conducted with Review Manager version 5.4 and R software using packages 'mada' and 'dmetatools'.

3. Result

3.1. Study selection

A total of 652 potential studies were identified after duplicates were removed. Of those, 583 were excluded during the screening phase, with 62 studies fully appraised. Seventeen studies [18–34] were included in this review (Fig. 1).

3.2. Study characteristics

Seventeen studies [18–34] comprising 1804 participants (AMI: n = 819, comparator: n = 985) from five countries were included from 2003 to 2022. Ten studies were conducted in China [21,22,24–28,31–33], three in Greece [18,19,23], two in Israel [29,34], one in Egypt [20] and one in Russia [30].

Different definitions of AMI were used. Seven studies [20,22,23,26,27,31,32] defined AMI based on the ESC and/or ACC and/or AHA guidelines. Two studies [18,28] used the universal definition of myocardial infarction. One study [19] included patients thrombolysed within 6 h of chest pain. One study [34] included coronary angiography evidence of coronary obstruction or thrombus. Two studies [24,25] established their own AMI criteria based on clinical symptoms, 12-lead ECG and positive cardiac biomarkers (cardiac troponin, Creatinine Kinase-MB (CK-MB) and myoglobin). One study [33] included AMI defined by the Chinese Medical Association. Three studies [21,29,30] did not document the parameters used for the definition of AMI. The main characteristics of the included studies are outlined in Table 1 and Table S6.

2.4. Risk of bias (quality) assessment

Methodological quality was independently assessed by ET and JZ using the Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) tool [13]. The QUADAS-2 tool estimates the risk of bias across the following domains: (1) Patient section; (2) Index test; (3) Reference standard, and (4) Flow and timing and applicability concerns across the following domains: (1) Patient selection; (2) Index tests and (3) Reference standard. Questionnaire details are available in Table S4.

Pre-analytical parameters can significantly affect the results of cfDNA quantification. DNA sampling and pre-processing factors were thoroughly assessed in all the included studies [14]. Five checkpoints were considered (1) the type of medium for analysis (plasma); (2) the type of sample tube; (3) if the sample was analysed within four hours following venepuncture; (4) the number and speed of centrifugation, and (5) Storage conditions before nucleic acid extracts (-20 °C or −80 °C freeze). Five points were deemed high-quality cfDNA processing, four to three were deemed acceptable quality, and two to zero were considered

extraction methods, cfDNA quantification methods, admission cfDNA concentration, cfDNA diagnostic test accuracy performance, the correlation between cfDNA level and cardiac biomarkers, and data relating cfDNA to complications post AMI were collected. When necessary, the authors of the included studies were contacted to provide additional details.

Table 2
Studies reporting cfDNA concentration in AMI group versus comparator group.

Author	Year	Number	cfDNA		cfDNA concentration (unit) on admission	p-value
			DNA extraction method	cfDNA quantification method		
Agiannitopoulos	2020	80 AMI 50 controls	QIAamp Circulating Nucleic Acid Kit (Qiagen)	Fluorometric quantification using Qubit® 3.0 fluorometer of Qubit™ ssDNA assay	AMI: 48.5 ng/µl (3.3)~ Controls: 8.2 ng/µl (0.3) ~	p < 0.05
				Fluorometric quantification using Qubit® 3.0 fluorometer of Qubit™ dsDNA assay	AMI: 6.7 ng/µl (0.3)~ Controls: 1.2 ng/µl (0.1) ~	p < 0.05
				NanoDrop® 1000 spectrophotometer for nucleic acid quantification, (the user selects an option to specify between RNA, DNA and ssDNA)	AMI: 22.0 ng/µl (1.2)~ Controls: 7.8 ng/µl (0.2) ~	p < 0.05
				probe-based qPCR using Rotor-Gene 6000 system with the TaqMan Copy Number Reference Assay TERT (This assay targets Telomerase reverse transcriptase (TERT) gene)	AMI: 42.8 ng/µl (2.9)~ Controls: 1.4 ng/µl (0.5) ~	p < 0.05
Antonatos	2006	13 AMI 30 controls	QIAamp Blood Kit (Qiagen)	probe-based? qPCR using **LightCycler**™ - reagent not reported	AMI: 6873 log GE/ml (357)' Controls: 4112 log GE/ml (234)'	p < 0.05
Arafat	2018	50 AMI 30 controls	QIAamp Min Elute Virus Spin kit (Qiagen)	SYBR-green mix dye-based qPCR with Low ROx (Primer: L1PA2 (90) using 96-well plates in a 7500 qPCR system	AMI: 179.3 ng/ml (151.1) Controls: 6.8 ng/ml (2.6)	P = 0.001
				SYBR-green mix dye-based qPCR with Low ROx (Primer: L1PA2 (222) using 96-well plates in a 7500 qPCR system	AMI: 114.1 ng/ml (83.3)' Controls: 3.2 ng/ml (1.8)	P = 0.001
Chang	2003	55 AMI 274 controls	QIAamp 96 Spin Blood DNA extraction Kit	Fluorometric quantification of PicoGreen® dsDNA assay (PicogGreen® reagent is a florescent probe that binds to dsDNA and create a nucleic acid stain by forming a highly luminescent complex, used for quantitating dsDNA)	AMI: 510 ng/ml (398)~ Controls: 36 ng/ml (23.8)~	NR
Cui	2013	49 AMI 60 controls	Not required	LMAX® microplate luminometer for the quantification of branched DNA-based Alu assay Duration for results to be obtained: 2-3 h	AMI: 5745 ng/ml (4013–8643)* Controls: 118 ng/ml (81–221)*	p < 0.05
Destouni	2009	47 AMI 100 controls	QIAamp DNA Blood MiniKit (Qiagen)	probe-based qPCR using **LightCycler**™ with a primer for the amplification of a 189 bp fragment of the beta-globin gene	AMI: 196 GE/ml (1.4–496212.1)* Controls: 31 GE/ml (6.5–266.1)*	p < 0.05
Jing	2011	22 AMI 60 controls	Not required	LMAX® microplate luminometer for the quantification of branched DNA-based Alu assay	AMI: 4439 ng/ml (2100.4–9435.5)* Controls: 117 ng/ml (80.8–218.6)*	p < 0.05
Lou	2015	120 AMI 60 controls	NR	SYBR-green mix dye-based qPCR with Alu-based qPCR assay (Primer size 201 bp) using an ABI 7500 sequence detector [Alu1] Running time required: 30 min	AMI: 4.2 log copies/ml (0.2)~ Controls: 1.8 log copies/ml (0.2)~	p < 0.05
				SYBR-green mix dye-based qPCR with Alu-based qPCR assay (Primer size 170 bp) using an ABI 7500 sequence detector [Alu2]	AMI: 5.4 log copies/ml (0.1)~ Controls: 2.9 log copies/ml (0.4)~	p < 0.05
				SYBR-green mix dye-based qPCR with Alu-based qPCR assay (Primer size 147 bp) using an ABI 7500 sequence detector [Alu3]	AMI: 5.1 log copies/ml (0.3)~ Controls: 1.7 log copies/ml (0.3)~	p < 0.05
				SYBR-green mix dye-based qPCR with Alu-based qPCR assay (Primer size 113 bp) using an ABI 7500 sequence detector [Alu4]	AMI: 7.0 log copies/ml (0.2)~ Controls: 2.9 log copies/ml (0.1)~	p < 0.05
				SYBR-green mix dye-based qPCR with Alu-based qPCR assay (Primer size 76 bp) using an ABI 7500 sequence detector [Alu5]	AMI: 7.21 log copies/ml (0.17)~ Controls: 3.79 log copies/ml (0.14)~	p < 0.05
				Alu = log (Alu1 + Alu2 + Alu3 + Alu4 + Alu 5)	AMI: 7.90 log copies/ml (0.17) Controls: 4.67 log copies/ml (0.24)~	p < 0.05
Qin	2016	38 AMI 32 controls	DNeasy Blood and Tissue Kit (Qiagen)	mtDNA was measured with a SYBR® green dye-based qPCR assay using an ABI PRISM 7300 sequence detection system. The primer sequence was the human NADH dehydrogenase 1 gene	AMI: 478 copies/µl (106)~ Controls: 157 copies/µl (97)~	p < 0.05
Rainer	2006	10 AMI 21 controls	QIAamp DNA Blood Kit (Qiagen)	probe-based qPCR analysis was performed using a PE Applied Biosystems 7700 sequence detector with a TaqMan probe for the beta-globin gene.	AMI: 660 kGE/l (440–1120)^ Controls: 350 kGE/l (250–610)^	p < 0.05

Table 2 (*continued*)

Author	Year	Number	cfDNA			p-value
			DNA extraction method	cfDNA quantification method	cfDNA concentration (unit) on admission	
Ren	2022	20 AMI 25 controls	QIAamp Circulating Nucleic Acid Kit (Qiagen)	cfDNA methylation analysis (genome wide method): cfDNA concentration quantification method is not specified.	AMI: 6.5 ng/ml Controls: 6.3 ng/ml	p = 0.21
		116 AMI 25 controls	QIAamp Circulating Nucleic Acid Kit (Qiagen)	cfDNA Methylation analysis (targeted method): cfDNA concentration quantification method is not specified. cfDNA underwent bisulfite conversion with EZ-96 Methylation Direct™ MagPrep. Bisulfite-converted cfDNA was interrogated using the TaqMan assay targeted at CORO6 locus as a heart-specific hypermethylation marker. **CORO6 ddPCR assay** was used to provide quantification of cardiomyocyte-specific cfDNA.	AMI: 1.0 copies/ml (0.8–2.0)* Controls: 0 copies / ml (0–0.9)*	P = 0.001
Shimony	2010	16 AMI 47 controls	Not required	Fluorometric quantification of SYBR®Gold Nucleic Acid Gel Stain (SYBR® Gold is a dye that exhibits florescence enhancement upon binding to nucleic acids (dsDNA or ssDNA or RNA)).	AMI: 735 ng/ml (350)~ Controls: 471 ng/ml (203)~	p < 0.05
Veiko	2008	7 AMI 18 controls	NR	Fluorometric quantification of Hoechst 33,528 nucleic acid stain (Hoechst 33,528 emits blue fluorescence when bound to dsDNA)	AMI: 862 ng/ml (608)~ Controls: 161 ng/ml (70)~	NR
Wang	2015	25 AMI 25 controls	QIAamp DNA blood Mini kit (Qiagen)	probe-based qPCR using **LightCycle®96** sequence detection system with primer for the beta-globin gene	AMI: 0.5 ng/μl (0.1)' Controls: 0.2 ng/μl (0.0)'	p < 0.05
				mtDNA was measured with probe-based qPCR using **LightCycle®96** sequence detection system with primer for the human NADH dehydrogenase 1 gene	AMI: 3.8 ng/μl (0.4)' Controls: 1.9 ng/μl (0.3)'	p < 0.05
Xie	2018	100 AMI 30 controls	QIAamp Circulating Nucleic Acid Kit (Qiagen)	Fluorometric quantification of PicoGreen® dsDNA assay (PicoGreen® dsDNA quantitation reagent is a florescent probe that binds to dsDNA and create a nucleic acid stain by forming a highly luminescent complex, used for quantitating dsDNA)	AMI: 5071 ng/mL (3709 – 6363)*$^€$ Controls: 974 ng/ml (328)~	p < 0.05+
Xu	2012	40 AMI 40 controls	Magnetic bead method	qPCR not otherwise specified	AMI: 253.6 ng/ml (45.7)~ Controls: 21.5 ng/ml (10.7)~	p < 0.05-
Zemmour	2018	31 AMI 83 controls	QIAsymphony Circulating DNA Kit	DNA Methylation analysis (targeted method): cfDNA measured using Qubit® dsDNA HS Assay Kit, followed by bisulfite conversion with EZ DNA Methylation Gold™ and PCR amplification. Bisulfite-treated cfDNA is interrogated using two methylation sensitive TaqMan™ probes to cover informative CpG sites in the FAMA1010A locus using prespecified primers. ddPCR™ Supermix for Probes was used and analysed with QuantaSoft analysis software to quantify cardiomyocyte specific cfDNA (**unmethylated FAM101A sequencing-based assay**)	AMI: 90.0 copies/ml (17.5–324.7)*$^€$ Controls: 16.0 copies/ml (−22.4 – 51.2)*$^€$	p < 0.05+

ddPCR: digital droplet Polymerase Chain Reaction, qPCR: Real-Time Polymerase Chain Reaction or Quantitative Polymerase Chain Reaction or quantitative real-time polymerase chain reaction (gives faster more detailed real time results used to quantify nucleic acid, NR: Not Reported, AMI - Acute Myocardial Infarction, STEMI – ST Elevated Myocardial Infarction, TnI Troponin I, cTnT cardiac troponin T, cTnI cardiac troponin I, CI: confidence Interval, KGE/l: kilogenome equivalents per liter, GE/ml: genomic equivalent per milliliter, ng/ml: nanograms per milliliter, ng/μL: nanograms per microliter, SE: standard error.
*median (interquartile range), 'mean (standard error), ~mean (standard deviation),ˆmedian (95% CI), +Mann-Whitney, - T-test.

4. Risk of bias within studies

4.1. QUADAS-2 methodological quality

Methodological quality varied. Most of the studies [18–20,22–29,31,32] excluded patients with comorbidities that could foreseeably raise cfDNA, which may have introduced selection bias. However, the patients included matched the review questions (i.e. no major concerns for the applicability domain). The interval between the index test and reference standard was sufficiently short to avoid changes in disease status in>80% of the studies, therefore having a low risk of bias for the flow and timing domain (Fig. 2 and Table S4).

4.2. cfDNA pre-analytical considerations for DNA processing

DNA processing quality was considered high quality in two studies [31,34], acceptable quality in ten studies [19,22–28,32,33] and poor quality in five studies [18,20,21,29,30] (Table S6).

5. Results of individual studies

5.1. Techniques for the detection of cfDNA

Eleven studies [18,19,21,23,26–28,31–34] applied cfDNA quantitative methods that required DNA to be extracted before cfDNA quantification. Various DNA extraction methods contribute to a broad range of cfDNA concentrations [35]. cfDNA can be detected by an array of quantitative techniques: (1) Optical technique - (i) Fluorometric quantification - Qubit™ ssDNA and dsDNA assay kit [18], PicoGreen® dsDNA assay [21,32], SYBR®Gold nucleic acid gel stain [29], Hoechst 33,528 nucleic acid stain [30] (ii) Spectrophotometric quantification - Nano-Drop® 1000 spectrometer for nucleic acid quantification [18] (iii) Luminometric quantification [22,24]; (2) quantitative polymerase chain reaction (qPCR) method (i) probe-based [18,19,23,27,31] (ii) dye-based [20,25,26] (iii) not otherwise specified [34] and (3) DNA methylation analysis [28,34]. Only two studies [28,34] inferred the origins of cfDNA and quantified cardiomyocyte-specific cfDNA concentrations (Table 2).

5.2. cfDNA concentration in AMI versus comparator group

From 17 studies [18–34] involving 1804 patients, cfDNA concentrations were substantially greater in AMI patients than in the
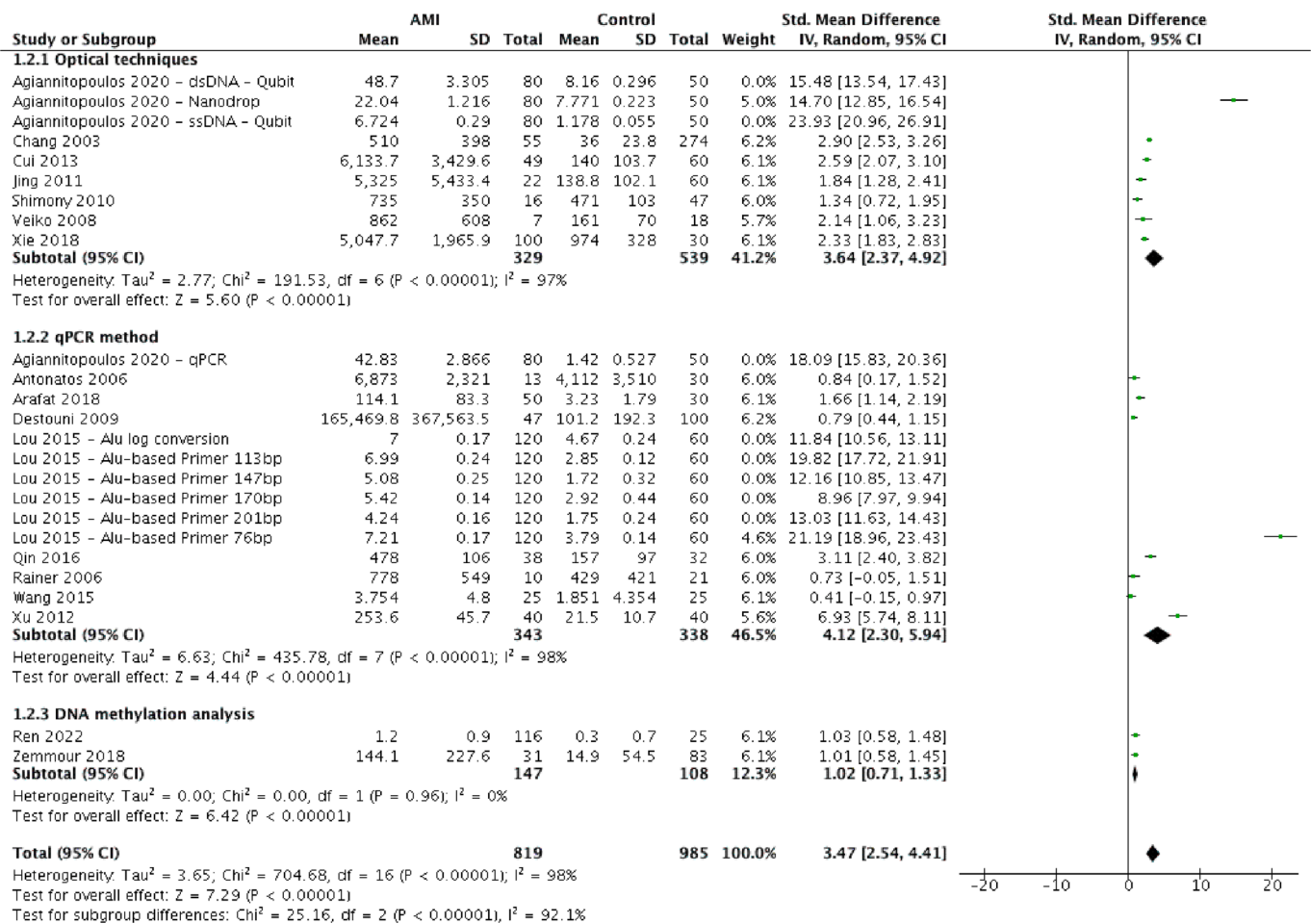
Study or Subgroup	AMI Mean	SD	Total	Control Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI	Std. Mean Difference IV, Random, 95% CI
1.2.1 Optical techniques									
Agiannitopoulos 2020 – dsDNA – Qubit	48.7	3.305	80	8.16	0.296	50	0.0%	15.48 [13.54, 17.43]	
Agiannitopoulos 2020 – Nanodrop	22.04	1.216	80	7.771	0.223	50	5.0%	14.70 [12.85, 16.54]	
Agiannitopoulos 2020 – ssDNA – Qubit	6.724	0.29	80	1.178	0.055	50	0.0%	23.93 [20.96, 26.91]	
Chang 2003	510	398	55	36	23.8	274	6.2%	2.90 [2.53, 3.26]	
Cui 2013	6,133.7	3,429.6	49	140	103.7	60	6.1%	2.59 [2.07, 3.10]	
Jing 2011	5,325	5,433.4	22	138.8	102.1	60	6.1%	1.84 [1.28, 2.41]	
Shimony 2010	735	350	16	471	103	47	6.0%	1.34 [0.72, 1.95]	
Veiko 2008	862	608	7	161	70	18	5.7%	2.14 [1.06, 3.23]	
Xie 2018	5,047.7	1,965.9	100	974	328	30	6.1%	2.33 [1.83, 2.83]	
Subtotal (95% CI)			329			539	41.2%	3.64 [2.37, 4.92]	
Heterogeneity: Tau² = 2.77; Chi² = 191.53, df = 6 (P < 0.00001); I² = 97%									
Test for overall effect: Z = 5.60 (P < 0.00001)									
1.2.2 qPCR method									
Agiannitopoulos 2020 – qPCR	42.83	2.866	80	1.42	0.527	50	0.0%	18.09 [15.83, 20.36]	
Antonatos 2006	6,873	2,321	13	4,112	3,510	30	6.0%	0.84 [0.17, 1.52]	
Arafat 2018	114.1	83.3	50	3.23	1.79	30	6.1%	1.66 [1.14, 2.19]	
Destouni 2009	165,469.8	367,563.5	47	101.2	192.3	100	6.2%	0.79 [0.44, 1.15]	
Lou 2015 – Alu log conversion	7	0.17	120	4.67	0.24	60	0.0%	11.84 [10.56, 13.11]	
Lou 2015 – Alu-based Primer 113bp	6.99	0.24	120	2.85	0.12	60	0.0%	19.82 [17.72, 21.91]	
Lou 2015 – Alu-based Primer 147bp	5.08	0.25	120	1.72	0.32	60	0.0%	12.16 [10.85, 13.47]	
Lou 2015 – Alu-based Primer 170bp	5.42	0.14	120	2.92	0.44	60	0.0%	8.96 [7.97, 9.94]	
Lou 2015 – Alu-based Primer 201bp	4.24	0.16	120	1.75	0.24	60	0.0%	13.03 [11.63, 14.43]	
Lou 2015 – Alu-based Primer 76bp	7.21	0.17	120	3.79	0.14	60	4.6%	21.19 [18.96, 23.43]	
Qin 2016	478	106	38	157	97	32	6.0%	3.11 [2.40, 3.82]	
Rainer 2006	778	549	10	429	421	21	6.0%	0.73 [-0.05, 1.51]	
Wang 2015	3.754	4.8	25	1.851	4.354	25	6.1%	0.41 [-0.15, 0.97]	
Xu 2012	253.6	45.7	40	21.5	10.7	40	5.6%	6.93 [5.74, 8.11]	
Subtotal (95% CI)			343			338	46.5%	4.12 [2.30, 5.94]	
Heterogeneity: Tau² = 6.63; Chi² = 435.78, df = 7 (P < 0.00001); I² = 98%									
Test for overall effect: Z = 4.44 (P < 0.00001)									
1.2.3 DNA methylation analysis									
Ren 2022	1.2	0.9	116	0.3	0.7	25	6.1%	1.03 [0.58, 1.48]	
Zemmour 2018	144.1	227.6	31	14.9	54.5	83	6.1%	1.01 [0.58, 1.45]	
Subtotal (95% CI)			147			108	12.3%	1.02 [0.71, 1.33]	
Heterogeneity: Tau² = 0.00; Chi² = 0.00, df = 1 (P = 0.96); I² = 0%									
Test for overall effect: Z = 6.42 (P < 0.00001)									
Total (95% CI)			819			985	100.0%	3.47 [2.54, 4.41]	
Heterogeneity: Tau² = 3.65; Chi² = 704.68, df = 16 (P < 0.00001); I² = 98%									
Test for overall effect: Z = 7.29 (P < 0.00001)									
Test for subgroup differences: Chi² = 25.16, df = 2 (P < 0.00001), I² = 92.1%									

Fig. 3. Forest plot of cfDNA levels in AMI versus the comparator group with subgroup analysis stratified according to the different cfDNA quantification methods.

comparator group (pooled SMD 3.47 (95% CI 2.54–4.41, $p < 0.001$)). The heterogeneity of these studies was high ($I^2 = 98\%$, $p < 0.001$). Subgroup analysis suggests that heterogeneity could partly be explained by the different cfDNA quantification methods used (Test for subgroup differences: Chi^2 25.16, $p < 0.001$, $I^2 = 92\%$, Fig. 3) but not explained by whether studies defined AMI according to consensus guidelines or if there was funding support (Fig. S1 and Fig. S2). The funnel plot for assessing publication bias was asymmetrical (Egger's test, $p < 0.001$, Fig. S3). On visual inspection, the inverted funnel shape poorly captured the distribution of included studies, with zero studies falling within the expected funnel region. Accordingly, publication bias could not be excluded.

5.3. Diagnostic performance of cfDNA in AMI

Six studies [18,22,25,28,32,34] with a total sample size of n = 804 (AMI: n = 496, comparator: n = 308) could be used for diagnostic performance. The overall diagnostic accuracy of cfDNA, when compared to the 'gold standard' in each study, yielded a sensitivity of 87% (95%CI 72%-95%), specificity of 96% (95%CI 92%-98%) and AUC of 0.96 (95% CI 0.93–0.98) (Fig. 4 and Table 3). Two studies [25,34] that did not use consensus guidelines for the diagnoses of AMI were removed as part of a sensitivity analysis. The four remaining studies [18,22,28,32] yielded a sensitivity of 89% (95%CI 62%-98%), specificity of 96% (95%CI 92%-99%) and AUC of 0.98 (95%CI 0.92——0.99) (Fig. S4). Cardiomyocyte-specific cfDNA studies [28,34] were removed as part of a sensitivity analysis. The remaining four studies [18,22,25,32] provided a pooled sensitivity, specificity and AUC of 90% (95%CI 86%-93%) and 98% (95%CI 95%-99%) and 0.97 (95%CI 0.88–0.99), respectively (Fig. S5).

Three studies [18,22,25] provided pooled estimates of sensitivity, specificity and AUC for cardiac troponins (cTnT [18], cTnI [22,25]) as 80% (95%CI 52%-94%), 97% (95%CI 86%-99%) and 0.96 (95%CI 0.78–0.99), respectively (Fig. S6).

5.4. Relationship between cfDNA and other cardiac biomarkers

Zemmour, et al. [34] described time to first increase of cfDNA concentrations within two hours from the onset of chest pain (similar to hs-cTn assays [36]), whereas conventional cTn assays only detects rises 4–10 h [4] after.Xie, et al. reported a significant association between cfDNA taken on admission and cTnI 6 h later (n = 100, Spearman correlation R = 0.57 (95%CI 0.41–0.69)) [32]. As expected, four studies [20–22,24] reported no relationship between admission cfDNA and troponin concentrations. When assessing the association between peak cfDNA and peak troponin concentrations, a strong association was described in two studies [22,29] (Shimony, et al.: Peak cfDNA and peak cTnT: r = 0.65 (95%CI 0.23–0.87), p = 0.006; Cui, et al.: peak cfDNA and peak cTnI: r = 0.72) (Table S7).

Peak cfDNA concentrations were significantly greater in patients with complicated post-AMI courses than those with an uncomplicated one [19,23] (Table S8). Similarly, Xie, et al. reported 1.8 fold greater cfDNA concentrations in the group experiencing one or more adverse events than the stable group (p < 0.001) [32].

5.5. Relationship between initial cfDNA and complicated admission

Rainer, et al. reported that admission cfDNA concentrations were two-fold greater in patients who developed heart failure (1060 vs 500
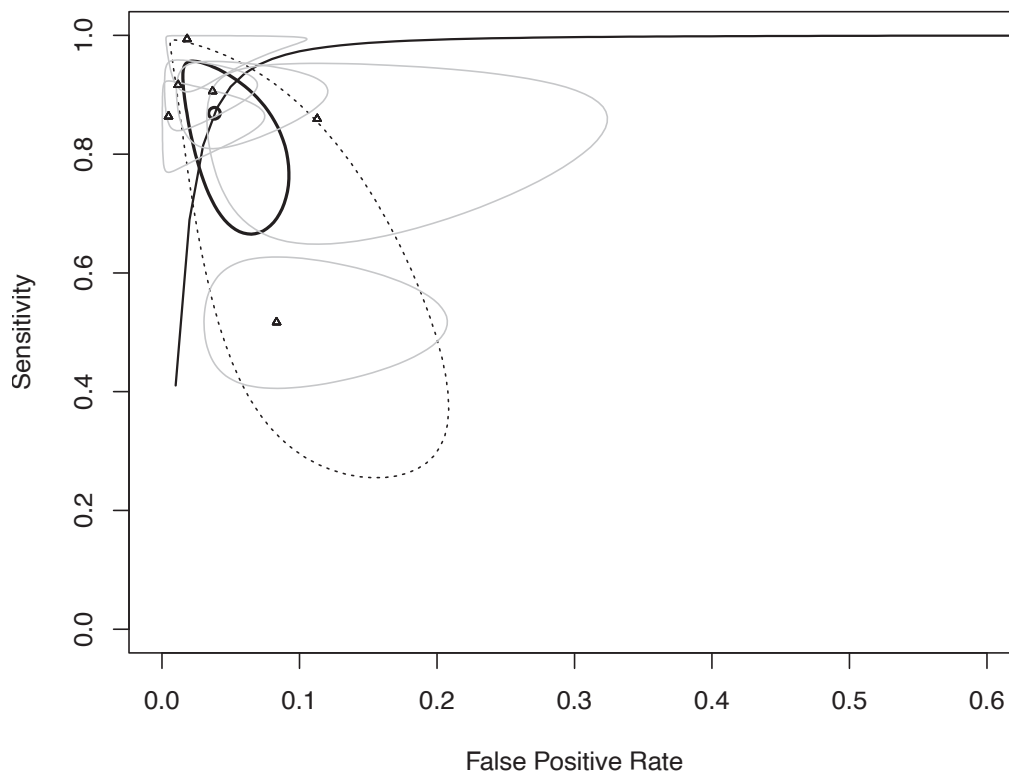
Fig. 4. Sroc of six studies with pooled estimates of auc, sensitivity and specificity for cfdna in the diagnosis of ami.

kGE/l, p = 0.009) and patients who later re-infarcted (1000 vs 530 kGE/l, p = 0.029); and three-fold greater in patients who had a cardiac arrest in that admission (1350 vs 525 kGE/l, p = 0.04) [27]. Similarly, Cui, et al. reported a significant difference in cfDNA concentration between the groups stratified according to the Global Registry of Acute Coronary Events (GRACE) scores (GRACE score < 100: median 1792 ng/ml (IQR 1198–3649); GRACE score 100–200: median 2635 ng/ml (IQR 2345–5220); GRACE score > 200: 8162 (IQR 2688–9206); p < 0.001) [22].

5.6. cfDNA and kidney impairment

None of the extracted studies included only CKD patients or reported outcomes in the subgroup of CKD patients with AMI.

6. Discussion

The main finding is that plasma cfDNA is an emerging biomarker of myocardial injury. Three major observations supported this concept. Firstly, cfDNA concentrations were more than three-fold greater in those patients presenting with AMI compared to a non-AMI comparator group. Secondly, some studies reported strong associations between peak cfDNA and cTn concentrations [22,29], suggesting that cfDNA increases due to cfDNA leak from necrotic cardiomyocytes during an AMI [32,37]. Thirdly, cfDNA demonstrated excellent diagnostic accuracy for AMI, performing similarly to conventional cTn and hs-cTn assays [5].

The latter observation is from the pooled sensitivity and specificity of cfDNA, which were 87% and 96%, respectively, extending improved sensitivity compared with conventional cTn assays but similar to hs-cTn assays [5]. This observation could attribute this higher sensitivity to the diagnosis of AMI in early presenters. cfDNA concentrations are increased within two hours from the onset of chest pain in those with AMI [8,34], whereas conventional cTn levels require 4–10 h after the onset of symptoms before concentrations increase [4].

The rapid increase in cfDNA concentrations from the onset of symptoms, similar to hs-cTn, is of major clinical relevance. Early identification of AMI facilitates earlier commencement of effective treatment, which is vital to reduce mortality [38]. Point-of-care hs-cTnI is currently being investigated to reduce time-to-invention compared to central laboratory processes [39]. While traditional DNA extraction methods were labour intensive and led to barriers to the rapid measurement of cfDNA; three included studies [24,25,29] described quantification methods without the need for prior DNA extraction. Point-of-care devices to measure cfDNA have been developed [40]. Prospective studies are needed to evaluate the accuracy and speed of these devices to determine AMI.

This study provides an expanded update to a 2015 mini-review [41]. In the previous review, two studies [30,33] before 2015 met the inclusion criteria but were not included. Comprehensive risks of bias were not assessed, and the definitions of AMI, which varied between studies, were not extracted or evaluated in the 2015 review; consequently, the potential impact of these factors on the conclusion remains uncertain. The present review was performed according to the PRISMA guidelines with risks of bias assessed using the QUADAS-2 tool and an inclusion of two pioneering studies that analysed the performance of cardiomyocyte-specific cfDNA as a biomarker of AMI.

Patients with kidney impairment have high mortality following AMI [42] but are less likely to receive invasive coronary angiography than those without [6]. The reason/s for this remains unclear, but there may be a potential bias against investigating patients with kidney disease [6,42]. Because hs-cTn concentrations are frequently chronically elevated in CKD, alternative diagnostic methodologies are vital. The clearance of cfDNA is mainly through the liver and spleen [3], and are not increased in patients with kidney dysfunction [9,10], as such, cfDNA is a potential diagnostic biomarker of AMI in patients with CKD. Hitherto, none of the included studies focused on CKD patients.

There remain two main challenges to the widespread use of cfDNA. First, interindividual variability due to various disease conditions (such as cancers [43], autoimmune diseases [44], solid organ transplants [45], sepsis [46] and trauma [15]) that can increase plasma cfDNA

Table 3

Studies reporting Diagnostic Test Accuracy data for cfDNA in AMI.

Author	Year	Number	Outcome to discriminate	cfDNA					Troponin			
				cfDNA quantification method	AUC (95%CI), p-value	Cut-off, units	Sensitivity	Specificity	AUC (95%CI)	Cut-off	Sensitivity	Specificity
Agiannitopoulos	2020	80 AMI 50 controls	AMI	Qubit® 3.0 ssDNA assay	0.99 (0.997–1.00), p < 0.001	20.45 ng/μl	100%	99%	0.94 (0.87–1.00), p < 0.001*	NR	NR	NR
				Qubit® 3.0 dsDNA assay	0.94 (0.89–0.98), p < 0.001	3.43 ng/μl	100%	98%				
				NanoDrop®	0.99 (0.99–1.00), p < 0.001	15.4 ng/μl	100%	92%				
				qPCR TaqMan Copy Number Reference Assay TERT	0.99 (0.99–1.00), p < 0.001	13.70 ng/μl	100%	99%				
Cui	2013	49 AMI 60 controls	ACS	branched DNA-based Alu assay	0.98 (0.96–1.00)	NR	92%	98%	0.74 (0.68–0.80)**	NR	57%	98%
			STEMI from ACS	branched DNA-based Alu assay	0.90 (0.84–0.95)	NR	NR	NR	0.76 (0.67–0.84)**	NR	NR	NR
Lou	2015	120 AMI 60 controls	AMI	Alu-based qPCR assay (Primer size 201 bp)	0.89 (0.82–0.95), p < 0.001	3.71 log copies/ml	77%	96%	0.90 (0.85–0.96), p < 0.001**	1.35 ng/ml	80%	84%
				Alu-based qPCR assay (Primer size 170 bp)	0.76 (0.66–0.85), p < 0.001	1.93 log copies/ml	100%	49%				
				Alu-based qPCR assay (Primer size 147 bp)	0.86 (0.79–0.93), p < 0.001	0.22 log copies/ml	100%	60%				
				Alu-based qPCR assay (Primer size 113 bp)	0.94 (0.89–0.99), p < 0.001	3.73 log copies/ml	87%	96%				
				Alu-based qPCR assay (Primer size 76 bp)	0.97 (0.93–1.00), p < 0.001	6.13 log copies/ml	87%	100%				
				Alu = log (Alu1 + Alu2 + Alu3 + Alu4 + Alu 5)	0.93 (0.87–0.99), p < 0.001	6.40 log copies/ml	92%	96%				
Ren	2022	116 AMI 25 controls	AMI	methylated CORO6 ddPCR assay	0.68 (0.59–0.78), p = 0.0037	NR	NR	NR	NR	NR	NR	NR
Xie	2018	100 AMI 30 controls	AMI	PicoGreen® dsDNA assay	0.96 (0.90–0.99)	110 ng	NR	NR	NR	NR	NR	NR
Zemmour	2018	31 AMI 83 controls	STEMI	demethylated FAM101A sequencing-based assay	0.94 (0.90–0.98), p < 0.001	NR	NR	NR	NR	NR	NR	NR

*Cardiac troponin T, **Cardiac Troponin I, NR: Not Reported.

concentrations. Therefore, DNA methylation analysis to determine cardiomyocyte-specific cfDNA represents a promising technique. In 2018, Zemmour, et al. reported the first cardiomyocyte-specific cfDNA marker, demethylated FAM101A, in diagnosing ST-elevation myocardial infarction. They reported excellent diagnostic accuracy [34]. Ren, et al. validated Zemmour's study and established another cardiomyocyte-specific cfDNA marker, methylated CORO6, which can provide an even more rapid result [28].

Second, the half-life of cfDNA ranges between several minutes and two hours [3], comparable with well-established markers of necrosis such as troponin [47] and CK-MB [48]. Nonetheless, biomarkers may persist for days despite their short biological half-life. Zemmour, et al. reported that after percutaneous coronary intervention, cfDNA concentrations increase rapidly before returning to baseline after one to two days. Meanwhile, cardiac troponin reduces over the next four to ten days [49] despite its short biological half-life [47]. The rapid change in cfDNA concentration should be considered an advantage to monitor the immediate treatment response and serve as a marker for adverse events after AMI. Nonetheless, time to dominant peak of plasma concentration cfDNA remains unclear and contradictory, indicating both earlier [8,26,28] and later [21,29,41] than that of troponin and CK-MB. Further studies are required to assess the kinetic pattern and molecular pathways [50] of cfDNA in AMI.

This systematic review and *meta*-analysis has some limitations inherent to the original studies. Eight included studies [19,21,24,25,29,30,33,34] applied a variety of methods to determine AMI. Given the lack of a single 'gold standard', the overall estimates of sensitivity or specificity may be biased, and it is not possible to determine whether the estimates would consistently overestimate or underestimate the true accuracy of cfDNA. There was also considerable heterogeneity in cfDNA extraction, cfDNA quantification and cut-off values used. It is worth noting that the development of a preferred quantification method to ensure standardisation is limited by several obstacles that include, but are not limited to, the lack of universal preanalytical standards, a growing range of pre-analytical methods, no standard reference materials and the need for further validation of quantification methodologies to demonstrate clinical relevance. While in-depth enquiry into these factors is beyond the scope of this review, at the moment, there is no optimal approach for the quantification of total cfDNA levels, and the development of such may be imperative [51]. Furthermore, most studies included in this analysis were diagnostic case controls, which introduce spectrum bias with the possibility of overestimating diagnostic performance. Publication bias also cannot be excluded with unpublished studies not included in this *meta*-analysis. Lastly, no studies investigated cfDNA concentration in CKD patients with AMI.

7. Conclusion

Plasma cfDNA appears to be a reliable biomarker of myocardial injury. Inferences from existing results are limited owing to methodology heterogeneity. Peak concentrations appear to correlate with poorer clinical outcomes. Further studies to assess point-of-care devices prospectively are required to fully evaluate the efficacy and accuracy of rapid on-site defections of AMI.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcha.2023.101246.

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