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Systematic Review on Circulating Cell Free DNA found in plasma of healthy controls

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ADMINISTRATIVE INFORMATION

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Review Stage at time of this submission - Risk of bias assessment.

Conflicts of interest - None declared.

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Amendments - This protocol was registered with the International Platform of Registered Systematic Review and Meta-Analysis Protocols (INPLASY) on 28 March 2025 and was last updated on 28 March 2025.

INTRODUCTION

Review question / Objective To establish ranges of circulating cell free DNA (cfDNA) levels in healthy patients based on the processing steps, and to make recommendations of methodologies that result in less variability, in order to inform and aid future early detection algorithms.
PICO:
P (Population): Healthy patients
I (Intervention): Measuring plasma cfDNA levels
C (Comparison): Primary data literature
O (Outcome): Informed means and standard deviations in ng/ml and recommended methods.

Rationale Circulating cell-free DNA (cfDNA) are nucleic acids measurable in liquid biopsies, commonly found in blood, urine, and various fluid collections including seminal, cerebrospinal, synovial, and early follicular fluid. These nucleic acids originate from lysed cells, and so circulating cfDNA refers to those that have translocated beyond their region of origin and are observed in the blood. In healthy individuals, homeostasis typically controls these levels through degradation, maintaining them at low levels under normal apoptotic conditions. However, in many disease states, including cancer, these safeguards are not only overwhelmed but cells also undergo apoptosis or necrosis at higher rates. As a result, significantly higher levels of cfDNA are found in the blood of diseased individuals compared to healthy individuals.

For disease monitoring, the most practical use of cfDNA analysis involves measuring these nucleic acids in whole blood samples from patients. Researchers typically choose between evaluating cfDNA levels in either blood serum or plasma, and plasma cfDNA is preferred for establishing sensitive biomarker guidelines, as it allows for a lower detection threshold. This underscores the need to develop robust quantification standards for plasma cfDNA in future experiments.

While many systematic reviews related to cfDNA concentrate on individuals with the disease, it is important to understand how technological steps impact the variability of cfDNA concentrations in healthy controls. This is especially important for

early detection strategies, where the ranges of healthy and diseased individuals may have a large overlap.

Condition being studied Healthy levels of plasma cfDNA.

METHODS

Search strategy Electronic Databases: Google Scholar, PubMed, Scopus, Embase.Search Terms: “cfDNA concentration” AND “healthy controls” AND “ng/ml” AND “plasma”.
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Participant or population Healthy people, usually healthy volunteers as controls for studies.

Intervention Measuring plasma cfDNA levels in plasma extracted from whole blood drawn from patients.

Comparator N/A.

Study designs to be included All study designs that include a healthy control group will be used, including but not limited to randomized control trials, cohort studies, and case-control studies.

Eligibility criteria
Exclude duplicates
Include only studies where plasma cfDNA is reported
Exclude studies without primary data
Include only studies on human patients
Exclude studies without a healthy control cohort
Include only studies where cfDNA concentrations are reported in ng/ml.
Include only studies that report means and standard deviations and/or individual level data.

Information sources Electronic Databases: Google Scholar, PubMed, Scopus, Embase.

Main outcome(s) While previous systematic reviews have targeted specific cancers or diseases to develop cfDNA assays as diagnostic biomarkers, this study offers a comprehensive analysis of cfDNA mean levels and standard deviations in healthy plasma, measured in ng/ml.

Additional outcome(s) The individual steps used to process cfDNA were also reviewed and evaluated to see which technical steps demonstrate the greatest contribution to variability based on the kits or methodologies employed.

Data management A shared google drive is used to store and manage Microsoft Excel, and Powerpoint, and Word documents.

Quality assessment / Risk of bias analysis The quality of the included studies will be assessed based on five categories: number of patients in the healthy cohort of the study (at least 10 or at least 30), blood collection (if the vial contained EDTA), the DNA isolation method (if a Quigen kit was used), DNA quantification method (if either PCR or QuiBit quantification), and the presence of individual patient level data.

The risk of bias will be evaluated using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) criteria (https://PubMed.ncbi.nlm.nih.gov/22007046/). The domains that will be assessed are patient selection, index test, reference standard, and flow and timing.

Strategy of data synthesis The following information will be extracted from all studies: (1) methods from collecting blood through cfDNA quantification: a) blood collection container b) cfDNA isolation methods c) cfDNA quantification methods (2) mean and standard deviations of concentrations of cfDNA in ng/ml (3) when available raw ng/ml data per volunteer sample.

Data for similar methodology flows will be aggregated and presented.

Subgroup analysis Multivariate analysis will be performed on subgroups to analyze the effect of specific technological steps on the subgroup variablity.

Sensitivity analysis Multivariate analysis will be performed to determine which step(s) have a statistically significant effect on the outcomes based on the methodology selection. This will help with overall recommendations.

Language restriction English.

Country(ies) involved United States.

Keywords cfDNA; plasma; healthy controls.

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