Quantifying DNA Lesions and Circulating Free DNA
Diagnostic Marker for Electropathology and Clinical Stage of AF

Pool, Lisa; van Wijk, Stan W.; van Schie, Mathijs S.; Taverne, Yannick J.H.J.; de Groot, Natasja M.S.; Brundel, Bianca J.J.M.

DOI
10.1016/j.jacep.2024.10.008

Publication date
2025

Document Version
Final published version

Published in
JACC: Clinical Electrophysiology

Citation (APA)
Pool, L., van Wijk, S. W., van Schie, M. S., Taverne, Y. J. H. J., de Groot, N. M. S., & Brundel, B. J. J. M. (2025). Quantifying DNA Lesions and Circulating Free DNA: Diagnostic Marker for Electropathology and Clinical Stage of AF. *JACC: Clinical Electrophysiology*, *11*(2), 321-332. https://doi.org/10.1016/j.jacep.2024.10.008

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 11, NO. 2, 2025

ORIGINAL RESEARCH

ATRIAL FIBRILLATION

Quantifying DNA Lesions and Circulating Free DNA

Diagnostic Marker for Electropathology and Clinical Stage of AF

Lisa Pool, MSc,[a,b] Stan W. van Wijk, MSc,[a] Mathijs S. van Schie, PhD,[b] Yannick J.H.J. Taverne, MD, PhD,[c] Natasja M.S. de Groot, MD, PhD,[b,d,*] Bianca J.J.M. Brundel, PhD[a,*]

ABSTRACT

BACKGROUND Atrial fibrillation (AF) persistence is associated with molecular remodeling that fuels electrical conduction abnormalities in atrial tissue. Previous research revealed DNA damage as a molecular driver of AF.

OBJECTIVES This study sought to explore the diagnostic value of DNA damage in atrial tissue and blood samples as an indicator of the prevalence of electrical conduction abnormalities and stage of AF.

METHODS High-sensitivity long-run real-time PCR was performed on mitochondrial (ND1) and nuclear (P53) DNA from atrial tissue samples from paroxysmal (PAF), persistent (PeAF), and longstanding persistent (LS-PeAF) AF, and sinus rhythm (SR) patients (n = 83). PicoGreen assay and quantitative polymerase chain reaction were used on circulating free DNA (cfDNA) markers (total cfDNA, β-globin, ND1, and P53) in blood samples of 70 patients with AF or SR. High-resolution epicardial mapping of the atria (n = 48) was conducted to quantify electrical conduction abnormalities.

RESULTS The number of DNA lesions gradually and significantly increased in PAF and PeAF and in patients with <3 years of AF compared with SR. In SR, the quantity of nuclear DNA damage significantly correlated with the proportion of fractionated potentials. Mitochondrial DNA lesions correlated with slower conduction velocity and lower potential amplitudes in AF samples. Also, mitochondrial cfDNA levels decreased in patients with >3 years of AF compared with <3 years of AF ($P = 0.004$).

CONCLUSIONS The quantity of DNA lesions in atrial tissue samples is associated with atrial conduction abnormalities and stage of AF. Serum DNA damage markers discriminate short- from long-term AF. Therefore, the quantity of DNA damage may have diagnostic value in clinical AF management. (JACC Clin Electrophysiol. 2025;11:321-332)

From [a]Physiology, Amsterdam Cardiovascular Sciences, Heart Failure, and Arrhythmias, Amsterdam University Medical Center, location Vrije Universiteit Amsterdam, Amsterdam, the Netherlands; [b]Translational Electrophysiology Unit, Department of Cardiology, Erasmus Medical Center, Rotterdam, the Netherlands; [c]Translational Cardiothoracic Research Laboratory, Department of Cardiothoracic Surgery, Erasmus Medical Center, Rotterdam, the Netherlands; and the [d]Department of Microelectronics, Circuits, and Systems, Faculty of Electrical Engineering, Mathematics, and Computer Sciences, Delft University of Technology, Delft, the Netherlands. *These authors have contributed equally to this work.
The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received July 5, 2024; revised manuscript received September 27, 2024, accepted October 4, 2024.

ISSN 2405-500X https://doi.org/10.1016/j.jacep.2024.10.008

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 11, NO. 2, 2025
FEBRUARY 2025:321–332

ABBREVIATIONS AND ACRONYMS

AF = atrial fibrillation

cfDNA = circulating free mitochondrial DNA

LAA = left atrial appendage

LORD-Q = long-run real-time polymerase chain reaction technique for DNA damage quantification

LS-PeAF = longstanding persistent atrial fibrillation

mtDNA = mitochondrial DNA

nDNA = nuclear DNA

PAF = paroxysmal atrial fibrillation

PeAF = persistent atrial fibrillation

qPCR = quantitative polymerase chain reaction

RAA = right atrial appendage

SR = sinus rhythm

Despite extensive research efforts, the optimal management strategy for atrial fibrillation (AF), the most prevalent cardiac arrhythmia, remains a challenge.[1,2] This challenge stems from an incomplete understanding of the molecular drivers of AF and the lack of effective diagnostic tools to assess the severity of AF pathology. Consequently, AF continues to pose an increased risk for stroke, heart failure, and mortality.[1,2] Although mobile health technology is rapidly developing, AF can be diagnosed only with the use of surface electrocardiography. Unfortunately, surface electrocardiography recordings do not provide comprehensive information on the severity of AF and therefore cannot be used to accurately assess the disease stage of AF. The limitations of the existing diagnostic methods hinder selection and development of personalized treatment modalities for AF. Therefore, the pressing need for improved diagnostics that may guide AF management remains.

So far, research has revealed that defects in specific molecular pathways underlie atrial conduction abnormalities that drive AF. Importantly, the severity of this so-called electropathology correlates with the stage of AF and may determine the response to AF treatment.[1-3] Identifying a molecular marker reflecting the stage of AF and the proportion of electropathology is expected to fuel the development of innovative personalized diagnostic tools that guide AF treatment strategies.

Previous experimental and clinical AF studies indicate that AF is intimately linked to DNA damage.[4,5] Based on this discovery, emerging research findings imply a role for DNA damage as a biomarker to stage AF. The levels of 8-hydroxy-2′-deoxyguanosine in atrial tissue and blood, serving as an indicator of oxidized proteins and DNA, offer diagnostic value for staging AF in the clinical practice, predicting AF recurrence after treatment, and anticipating the onset of postoperative AF.[6] Moreover, circulating free mitochondrial DNA (cfDNA) levels discriminate between the various stages of AF, substantiating a role for cfDNA as a potential biomarker for clinical AF diagnostics.[7] So far, no studies have investigated whether the quantity of DNA lesions represent a biomarker for the clinical stage of AF and severity of electropathology.

Recently, Lehle et al[8] developed a reliable long-run real-time quantitative polymerase chain reaction method for assessing the number of DNA lesions (LORD-Q). The principle relies on the presence of DNA lesions in the DNA template that disrupt the polymerase actions, resulting in impaired DNA synthesis and consequently a reduced polymerase chain reaction (PCR) product.[8] LORD-Q is highly sensitive for measuring lesions in mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) in cells and human tissue,[9-11] making it a reliable method for assessing the quantity of DNA lesions in clinical AF atrial tissue samples. The present study was designed to assess: 1) the quantity of DNA lesions in atrial tissue samples of patients staged according to current guidelines[12] into paroxysmal AF (PAF), persistent AF (PeAF), and longstanding persistent AF (LS-PeAF); 2) the correlation between the quantity of DNA lesions and proportion of conduction abnormalities measured during sinus rhythm (SR); and 3) the validity of blood-based DNA damage markers to stage the severity of AF.

METHODS

Details on all methods are documented in the Supplemental Methods.

RESULTS

STUDY SAMPLES. Baseline characteristics of the enrolled patients (n = 83) are summarized in **Table 1**. Patients underwent coronary artery bypass grafting, mitral, aortic, and/or tricuspid repair or replacement, a combination of coronary artery bypass grafting and valvular repair or replacement, or correction for a congenital heart defect. Patients were allocated to the SR (n = 20) or AF (n = 63) group, the latter including PAF (n = 30), PeAF (n = 18), and LS-PeAF (n = 15). The median duration since AF diagnosis was 36 months (Q1-Q3: 10.5 months to 7.4 years). For further details, see the Supplemental Results.

NUMBER OF DNA LESIONS RELATES TO THE STAGE OF AF. To quantify the number of DNA lesions in atrial tissue samples, LORD-Q was first validated by subjecting atrial tissue DNA samples to UV radiation (254 nm) for either 10, 30, or 60 minutes at 20 mJ/cm^2. DNA lesions were already robustly quantifiable after 10 minutes of UV radiation, as reflected by a 5-fold increase compared with atrial DNA samples without UV radiation (Supplemental Figure 1). The dose-dependent increase in DNA lesions after 10 minutes (15.33 lesions per 10 kb), 30 minutes (24.94 lesions per 10 kb), and 60 minutes (34.45 lesions per 10 kb) UV radiation reveals that the LORD-Q method is sensitive for the detection of the number of DNA lesions in atrial tissue samples.

TABLE 1 Patient Characteristics

	SR (n = 20)	PAF (n = 30)	PeAF (n = 18)	LS-PeAF (n = 15)	AF, Total (n = 63)	*P* Value
Tissue samples						
RAA	20	20	10	11	41	
LAA	0	10	11	6	27	
Serum samples	19	24	14	13	51	
Age, y	63.3 ± 12.4	66.9 ± 12.5	64.7 ± 10.6	72.3 ± 7.5	67.5 ± 11.2	0.099
Male	14 (70)	23 (76.7)	13 (72.2)	14 (93.3)	50 (78.1)	0.383
Underlying heart disease						0.013[a]
IHD	8 (40)	4 (13.3)	0 (0)	4 (26.7)	8 (50)	
VHD	2 (10)	16 (53.3)	12 (66.7)	8 (53.3)	36 (94.7)	
IHD/VHD	5 (25)	5 (16.7)	1 (5.6)	2 (13.3)	8 (61.5)	
CHD	5 (25)	5 (16.7)	4 (22.2)	1 (6.7)	10 (66.7)	
Cardiovascular risk factors						
Hypertension	12 (60)	18 (60)	7 (38.9)	10 (66.7)	35 (74.5)	0.369
Hypercholesterolemia	4 (20)	10 (33.3)	6 (33.3)	4 (26.7)	20 (83.3)	0.737
Diabetes mellitus	2 (10)	4 (13.3)	2 (11.1)	3 (20)	9 (81.8)	0.837
Thyroid disease	0 (0)	1 (3.3)	1 (5.6)	0 (0)	2 (100)	0.630
Myocardial infarction	6 (70)	5 (16.7)	1 (5.6)	2 (13.3)	8 (57.1)	0.237
Left ventricular function						0.324
Normal	18 (90)	21 (70)	14 (77.8)	10 (66.7)	45 (71.4)	
Mild	2 (10)	9 (30)	4 (22.2)	5 (33.3)	18 (90)	
Body mass index						0.111
Normal	6 (30)	15 (50)	5 (27.8)	2 (13.3)	22 (78.6)	
Overweight	9 (45)	13 (43.3)	8 (44.4)	7 (46.7)	28 (75.7)	
Obese	5 (25)	2 (6.7)	5 (27.8)	6 (40)	13 (72.2)	
Antiarrhythmic drugs						
Class I	0 (0)	1 (3.3)	1 (3.3)	0 (0)	2 (100)	0.630
Class II	14 (70)	16 (53.3)	9 (50)	12 (80)	37 (72.5)	0.201
Class III	0 (0)	7 (23.3)	4 (22.2)	0 (0)	11 (100)	0.079
Class IV	2 (10)	0 (0)	1 (5.6)	1 (6.7)	2 (50)	0.209
Digoxin	0 (0)	6 (20)	5 (27.8)	2 (13.3)	13 (100)	0.107
Duration AF, y						
<3	-	11	8	1	-	
>3	-	10	4	11	-	

Values are n, n (%), or mean ± SD. [a]$P < 0.05$, SR vs type of AF (Pearson chi-square test and Yates' continuity correction).
CHD = congenital heart disease; IHD = ischemic heart disease; VHD = valvular heart disease.

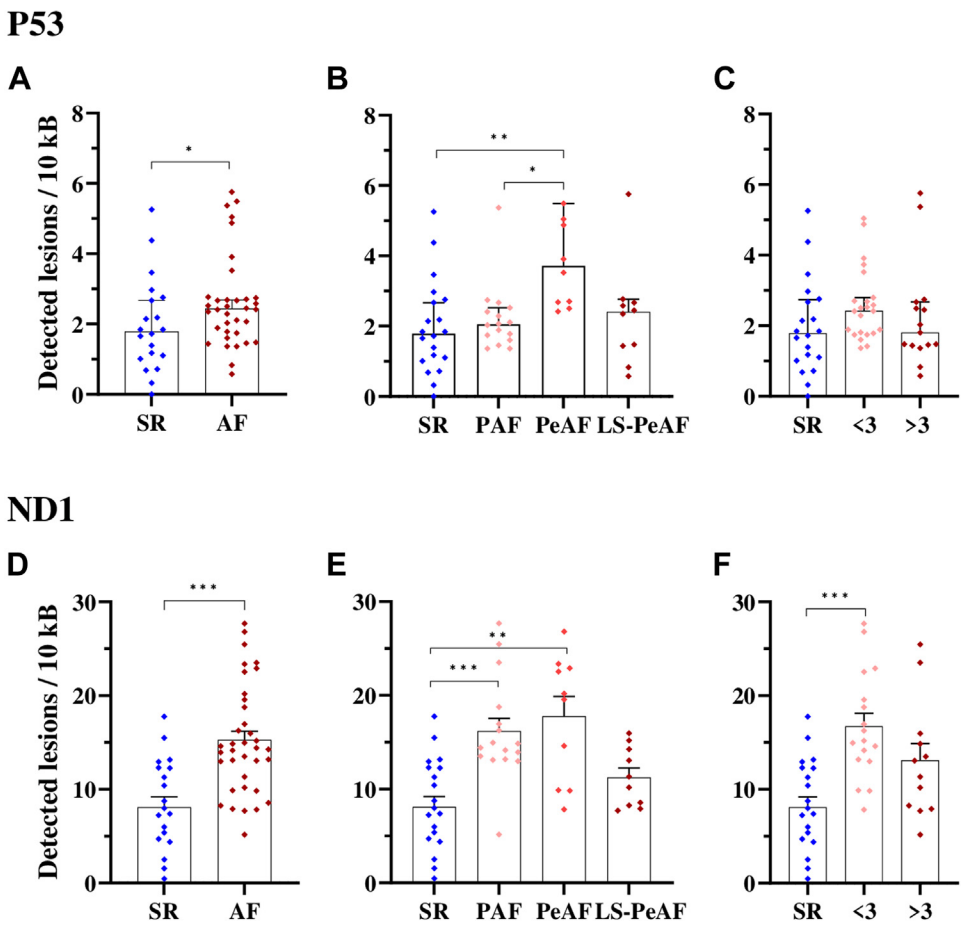
Next, the number of DNA lesions in atrial tissue samples was determined in samples from AF and SR patients. As shown in Figure 1, the number of nDNA lesions (P53) per 10 kb in the right atrial appendage (RAA) was significantly higher in AF compared with SR patients: (SR: 1.78 [Q1-Q3: 1.03-2.74] vs AF: 2.44 [Q1-Q3: 1.75-2.77]; $P = 0.047$). In addition, the number of nDNA lesions was significantly higher in PeAF compared with SR (1.78 [Q1-Q3: 1.03-2.74] vs 3.72 [Q1-Q3: 2.64-5.15]); $P = 0.002$). Among AF patients, nDNA lesions were higher in PeAF compared with PAF patients (2.05 [Q1-Q3: 1.64-2.49] vs 3.72 [Q1-Q3: 2.64-5.15]; $P = 0.02$).

For the mitochondrial genome (ND1), the number of DNA lesions per 10 kb in the RAA was significantly increased in AF compared with SR patients (SR: 8.09 ± 1.13 vs AF: 15.25 ± 0.97; $P < 0.001$) (Figure 1). DNA lesions increased in PAF (16.19 ± 1.37) and PeAF (17.76 ± 2.12) compared with SR (8.09 ± 1.13) (both $P \leq 0.002$). A similar trend was observed for nDNA and mtDNA in the left atrial appendage (LAA) and for the mitochondrial gene AS2, as shown in Supplemental Figures 2 and 3, suggesting overall genomic instability. No significant differences were observed between the quantity of DNA lesions in relation to the underlying heart disease in the various AF groups.

DNA LESIONS INCREASE WITHIN THE FIRST 3 YEARS AFTER AF DIAGNOSIS. AF patients were classified as short-term (<3 years) or long-term AF (>3 years) if the date of AF diagnosis was available. As shown in Figure 1F, the number of mtDNA lesions (ND1) increased significantly in patients with <3 years AF

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 11, NO. 2, 2025
FEBRUARY 2025:321–332

FIGURE 1 DNA Lesions per 10 kb in Nuclear and Mitochondrial Genome



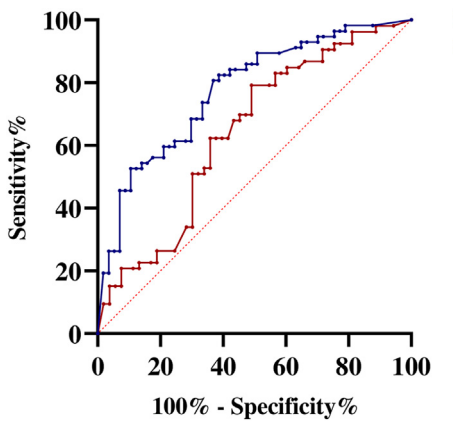
(Top) nuclear DNA (nDNA) lesions (P53). (Bottom) mitochondrial DNA (mtDNA) lesions (ND1). DNA lesions are presented in (A and D) sinus rhythm (SR) and atrial fibrillation (AF) patients; (B and E) SR, paroxysmal atrial fibrillation (PAF), persistent atrial fibrillation (PeAF), and longstanding persistent atrial fibrillation (LS-PeAF) patients; and (C and F) SR and patients with <3 years and >3 years of AF. $P < 0.05$ is considered to be significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

since diagnosis compared with SR ($P < 0.001$). The median number of mtDNA lesions per 10 kb was 8.09 ± 1.13 in SR patients and 16.72 ± 1.39 in patients with <3 years AF since diagnosis (**Figure 1**).

DIAGNOSTIC VALUE OF DNA LESIONS IN RIGHT ATRIAL TISSUE. To calculate the discriminative power of the quantity of DNA lesions, receiver operating characteristic (ROC) curves were constructed based on the number of mtDNA and nDNA lesions detected in RAA in all stages of AF vs SR. As shown in **Figure 2**, the areas under the receiver operating characteristic curve (AUC) were 68% and 82% for P53 and ND1, respectively. The cutoff point was 1.86 for the nuclear marker P53, which corresponds to a sensitivity of 78% and a specificity of 60% ($P = 0.019$). The cutoff point was 13.03 for the mitochondrial marker ND1, which corresponds to a sensitivity of 69% and a specificity 85%. The ROC curve of the mitochondrial marker AS2 is shown in the Supplemental Figure 3, with the similar result of an AUC of 63%.

Because a reduction in DNA lesions was observed in LS-PeAF, ROC curves were constructed without those patients. The AUCs improved considerably: to 71% and 87% for P53 and ND1, respectively, as shown in Supplemental Figure 4A. In addition, ROC curves were constructed for AF patients with <3 years vs >3 years of AF since diagnosis (Supplemental Figure 4B), showing AUCs of 67% and 62% for P53 and ND1, respectively. The respective AUCs were 73% and 86%

FIGURE 2 Receiver-Operating Characteristic Curves of DNA Damage



Diagnostic value of nDNA (P53) and mtDNA (ND1) damage levels in classifying AF. ROC curves with areas under the curve of 68% and 82% for P53 (red) and ND1 (blue), respectively. Abbreviations as in Figure 1.

for patients with >3 years AF and 64% and 79% for patients with <3 years AF, as shown in Supplemental Figures 4C and 4D. These findings indicate that the quantity of DNA lesions have value in discriminating between SR and AF patients, especially PAF and PeAF, and therefore may represent a diagnostic biomarker for AF.

NUMBER OF DNA LESIONS CORRELATES WITH THE PROPORTION OF ELECTRICAL CONDUCTION ABNORMALITIES. To correlate the number of nDNA and mtDNA lesions with the proportion of electrical conduction abnormalities during SR, a total of 52,282 potentials from RAA of SR patients (n = 18) and RAA or LAA of AF patients (n = 30) were included for analysis. As shown in **Figure 3A**, the number of nDNA lesions is primarily associated with fractionation in SR patients only, whereas the number of mtDNA lesions is associated with conduction velocity in AF patients (Supplemental Table 1, Supplemental Figure 5).

As shown in **Figure 3B**, a higher number of nDNA lesions was associated with a higher number of double and fractionated potentials in SR patients. In addition, single potentials were less frequently recorded when the number of DNA lesions was high. These findings indicate that nDNA lesions represent a potential marker for fractionation in SR patients.

On the other hand, for the number of mtDNA lesions, a significant inverse correlation with conduction velocity in AF patients (**Figure 3C**), particularly in patients with short-term AF (<3 years since diagnosis) was observed (**Figure 3D**). Also, the median potential voltages and the number of mtDNA lesions correlated inversely in patients with <3 years of AF since diagnosis (**Figure 3E**). These findings indicate that the number of mtDNA lesions is associated with the proportion of electrical conduction disorders measured during SR in AF patients. All correlations between DNA lesions and electrical parameters are listed in Supplemental Table 1.

SERUM-LEVEL DNA DAMAGE MARKERS IN SR AND AF PATIENTS. PicoGreen assay was used as a measure to detect the total DNA concentration, a marker for DNA damage,[13-15] in serum samples, as shown in **Figure 4A**. These measurements were performed in patients with SR (n = 19), PAF (n = 24), PeAF (n = 14), and LS-PeAF (n = 13), and in control subjects (n = 21). Compared with control samples, the concentration of total cfDNA (PicoGreen) was higher in SR (0.39 ± 0.34 ng/mL vs 0.13 ± 0.10 ng/mL; $P = 0.036$). No differences were observed in the concentration of cfDNA between AF and SR patients or among SR, PAF, PeAF and LS-PeAF patients.

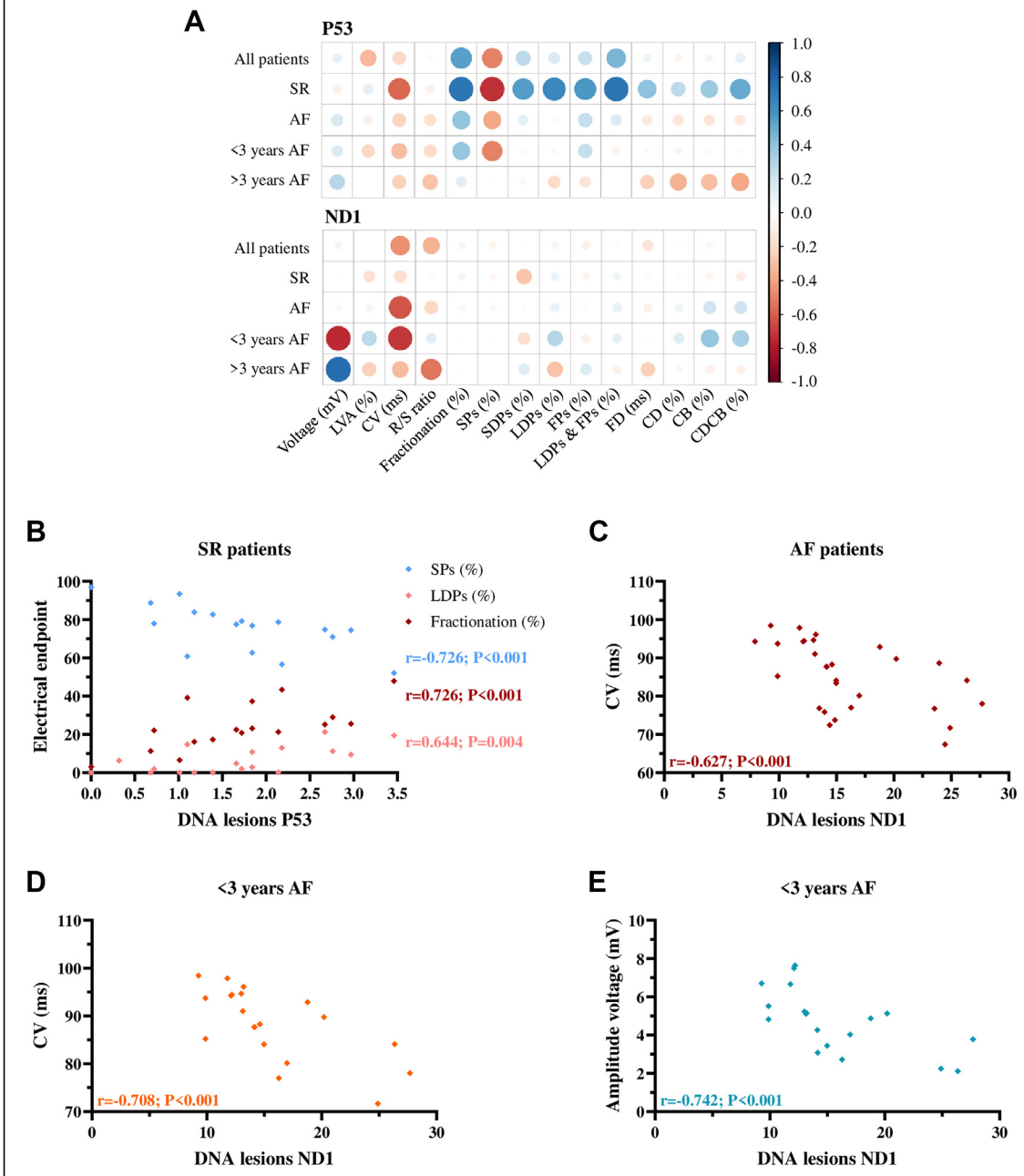
For β-globin (**Figure 4B**) and P53 (**Figure 4C**), serum DNA concentrations increased in SR patients compared with control subjects (both $P < 0.05$). No differences in the concentration of serum DNA were observed when the patients were classified according to the stages of AF (**Figures 4B and 4C**). However, patients with <3 years of AF since diagnosis had a mean concentration of 2,275.06 ± 622.31 copies/mL for ND1 (**Figure 4**), and this was higher than in patients with >3 years of AF (817.79 ± 195.26; $P = 0.004$), indicating that the mitochondrial serum DNA was lower in long-term AF patients compared with short-term AF (**Figure 4D**).

SERUM cfDNA AS DIAGNOSTIC MARKER FOR AF. Serum DNA markers significantly correlated with each other, as shown in **Figure 5**. Moderate correlation was observed between ND1 and β-globin ($r = 0.489$; $P < 0.001$), whereas the serum markers P53, β-globin, and total cfDNA (PicoGreen) revealed a close to perfect correlation ($r > 0.95$; $P < 0.001$). Similar results were found for the mitochondrial markers ND1 and AS2 ($r = 0.896$; $P < 0.001$) (Supplemental Figure 6).

To determine whether serum cfDNA markers have discriminative power for classifying AF, ROC curves were constructed for the serum markers total cfDNA (PicoGreen), P53, β-globin, and ND1. Serum markers have limited power to discriminate between SR and AF patients, as shown in Supplemental Figure 7. Nevertheless, serum DNA markers discriminate between patients with <3 years and >3 years of AF after diagnosis, as shown in **Figure 6**. The AUCs were 69%,

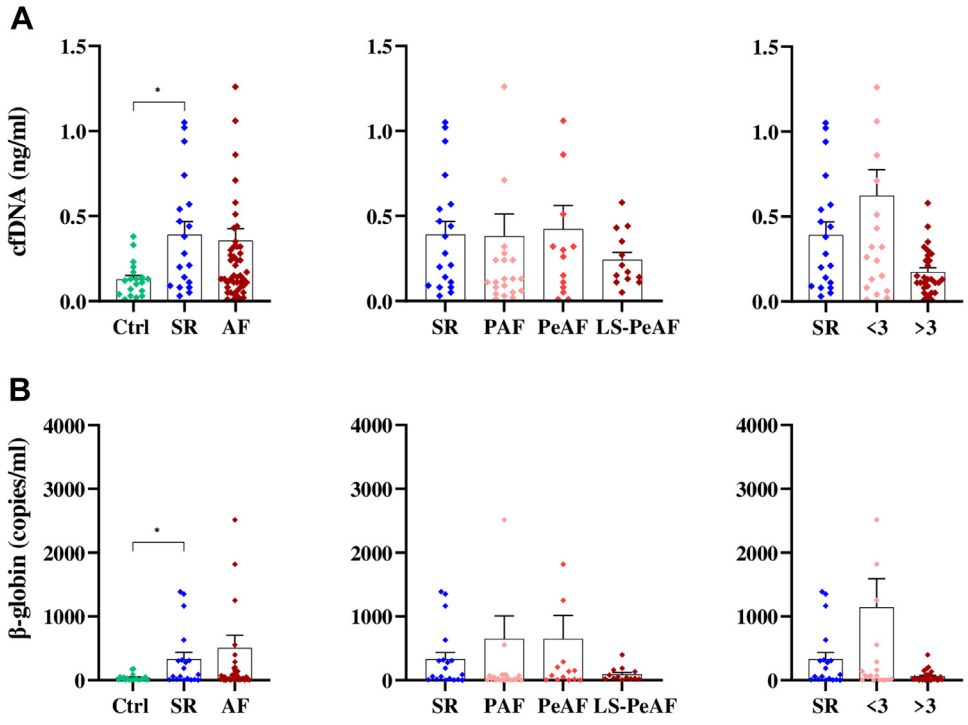
JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 11, NO. 2, 2025
FEBRUARY 2025:321–332

FIGURE 3 Correlogram and Scatterplots Between nDNA (P53) and mtDNA (ND1) Lesions and Electrical Parameters Measured in Right and Left Atrial Appendage Samples



(A) Correlation coefficients (*r*) of nDNA (P53) and mtDNA (ND1) lesions with electrical parameters. (B) Significant correlations were observed between nDNA lesions and single potentials (SPs) (n = 17; $r = -0.726$; $P < 0.001$), long double potentials (LDPs) (n = 18; $r = 0.644$; $P = 0.004$) and fractionation (n = 17; $r = 0.726$; $P < 0.001$) in SR patients. (C) In AF patients, significant correlation was observed between mtDNA lesions and conduction velocity (CV) (n = 29; $r = -0.627$; $P < 0.001$). (D) In patients with <3 years AF, significant correlation between mtDNA lesions and CV (n = 20; $r = -0.708$; $P < 0.001$). (E) Significant correlation between mtDNA lesions and median potential voltages (n = 19; $r = -0.742$; $P < 0.001$). CB = conduction block; CD = conduction delay; CDCB = conduction delay and block; CV = conduction velocity; FD = fractionation duration; FP = fractionation potential; LVA = low voltage area; SDP = short double potential; other abbreviations as in Figure 1.

FIGURE 4 Serum Circulating Fee DNA (cfDNA) Marker Levels in SR and AF Patients



Serum DNA concentration of (A) total cfDNA (PicoGreen), (B) β-globin, (C) P53, and (D) ND1 in control subjects and SR and AF patients (PAF, PeAF, LS-PeAF, <3 years AF, and >3 years AF). *$P < 0.05$. Abbreviations as in Figure 1.

75%, and 73% for, respectively, β-globin, P53, and total cfDNA (PicoGreen); corresponding to sensitivities of 48%, 63%, and 63% and specificities of 100%, 90%, and 84%, respectively. For the mitochondrial marker (ND1) the AUC was 83%, which corresponds to sensitivity of 79% and specificity of 84%. This indicates that serum markers for cfDNA have value in discriminating between patients with short-term and long-term AF. The mitochondrial marker AS2 is added in Supplemental Figure 7.

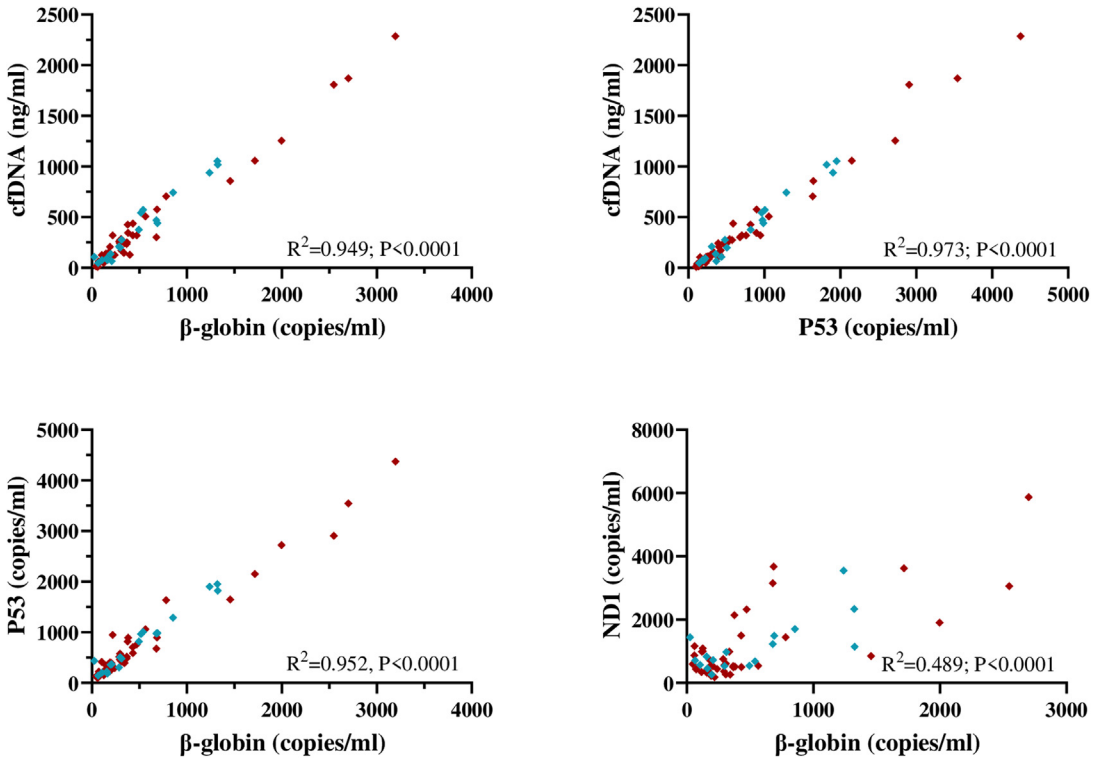
VALIDATION IN AN INDEPENDENT AF COHORT. To validate whether serum cfDNA markers have discriminative power for classifying AF, serum samples from an independent cohort were tested for β-globin levels. Here, serum samples from patients receiving AF treatment by pulmonary vein isolation (PVI) or electrocardioversion (ECV) were included (Supplemental Tables 2 and 3). In the PVI/ECV group, serum levels of β-globin were significantly higher in patients with PAF and PeAF compared with control subjects ($P < 0.001$) (Figure 7). Interestingly, β-globin levels were higher in PeAF patients with AF recurrence after AF treatment compared with PeAF patients without recurrence ($P = 0.05$) (Figure 7).

DISCUSSION

Although AF has been associated with increased DNA damage in experimental and human atrial tissue samples, the value of DNA damage as a quantitative marker to stage the proportion of conduction abnormalities and, as such, the severity of AF, has not been established. In the present study, we used LORD-Q to detect a significant and gradual increase in the number of DNA lesions in both PAF and PeAF patients and in patients with <3 years AF since diagnosis (short-term AF) compared with SR. Taking these results together, LORD-Q is a robust and reliable method for DNA lesion quantification in atrial tissue samples, and the quantity of DNA damage may represent a diagnostic biomarker for AF staging. Moreover, this study shows that the number of DNA lesions correlates with electrical parameters. In depth, lesions in the nuclear genome represent a potential marker for fractionation in SR patients, and lesions in the mitochondrial genome are associated with the proportion of electrical conduction disorders in AF patients. This finding is substantiated by the observation that mitochondrial serum DNA has diagnostic value in

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 11, NO. 2, 2025
FEBRUARY 2025:321–332

FIGURE 5 Correlation Between DNA Serum Markers in SR (Blue) and AF (Red) Patients

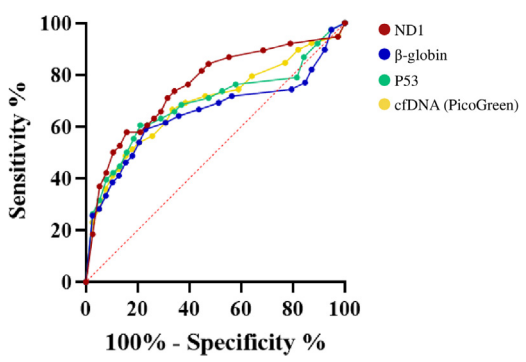


Significant and strong correlations were observed among total cfDNA (PicoGreen), P53, β-globin, and ND1. The strongest correlations were observed between total cfDNA (PicoGreen) and P53 ($r = 0.973$), total cfDNA (PicoGreen) and β-globin ($r = 0.949$), and P53 and β-globin ($r = 0.952$). Moderate correlation was observed between β-globin and ND1 ($r = 0.489$) (all $P < 0.0001$). Abbreviations as in Figures 1 and 4.

discriminating short-term AF from long-term AF. Because the clinical stage of AF is most likely a major determinant in antiarrhythmic therapy effectiveness, quantification of DNA damage levels may also aid in therapy selection (Supplemental Tables 4-8).[16,17]

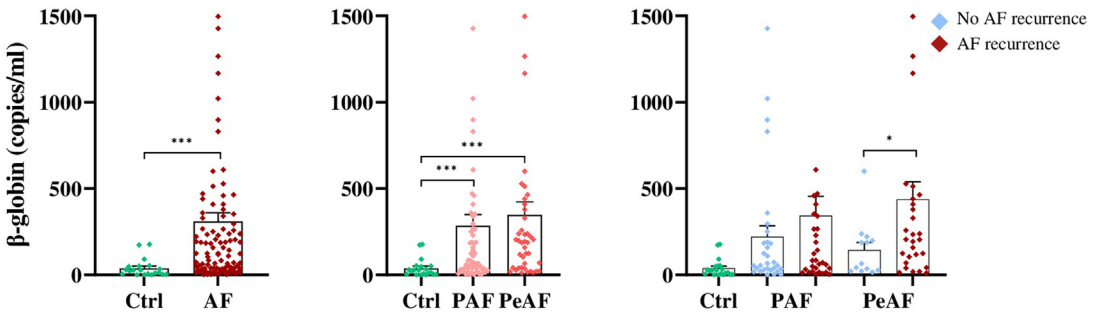
QUANTIFICATION OF DNA LESIONS IN AF. Compared with SR, the number of DNA lesions was significantly increased in PAF and PeAF, whereas no difference was observed in LS-PeAF. This observation may imply that with AF progression, a different pathophysiologic mechanism is induced, which is supported by experimental study findings. In a goat model of AF, 16 weeks of sustained AF was associated with (ultra)structural adaptations in atrial cardiomyocytes resembling hibernation.[18] AF-induced hibernation in atrial cardiomyocytes was characterized by degradation of the sarcomere structure (myolysis) and the presence of pale nuclei, indicating decondensed chromatin, a marker of DNA damage.[19-21] Hibernation of cardiomyocytes was described as a form of

FIGURE 6 ROC Curve Serum DNA Concentration



Diagnostic value of serum DNA in patients with <3 years and >3 years AF. ROC curve with an area under the curve of was 69%, 75% and 73% for respectively β-globin, P53 and total cfDNA (PicoGreen). For the mitochondrial marker ND1 was the AUC respectively 83%. Abbreviations as in Figures 1, 2, and 4.

FIGURE 7 DNA Serum Levels of β-Globin in the PVI/ECV Group



Concentration of β-globin in PAF and PeAF patients with or without AF recurrence after PVI/ECV. Data are mean ± SEM, and $P < 0.05$ is considered to be significant; ***$P \leq 0.0001$. Abbreviations as in Figure 1.

programmed cell survival by dampening the activity of the cell[22] and mitigation of further DNA damage to ultimately promote cell survival.[23] In line with the findings from the goat model of AF persistence, hibernation was also observed as a key structural feature in atrial tissue samples of patients with (longstanding) PeAF,[24,25] suggesting that longer periods of AF initiate a different pathophysiologic mechanism involving inactivity of cardiomyocytes to minimize further damage. Our data support these previous findings, as we also observed an increase in DNA damage only in patients with PAF and PeAF and not in those with LS-PeAF.

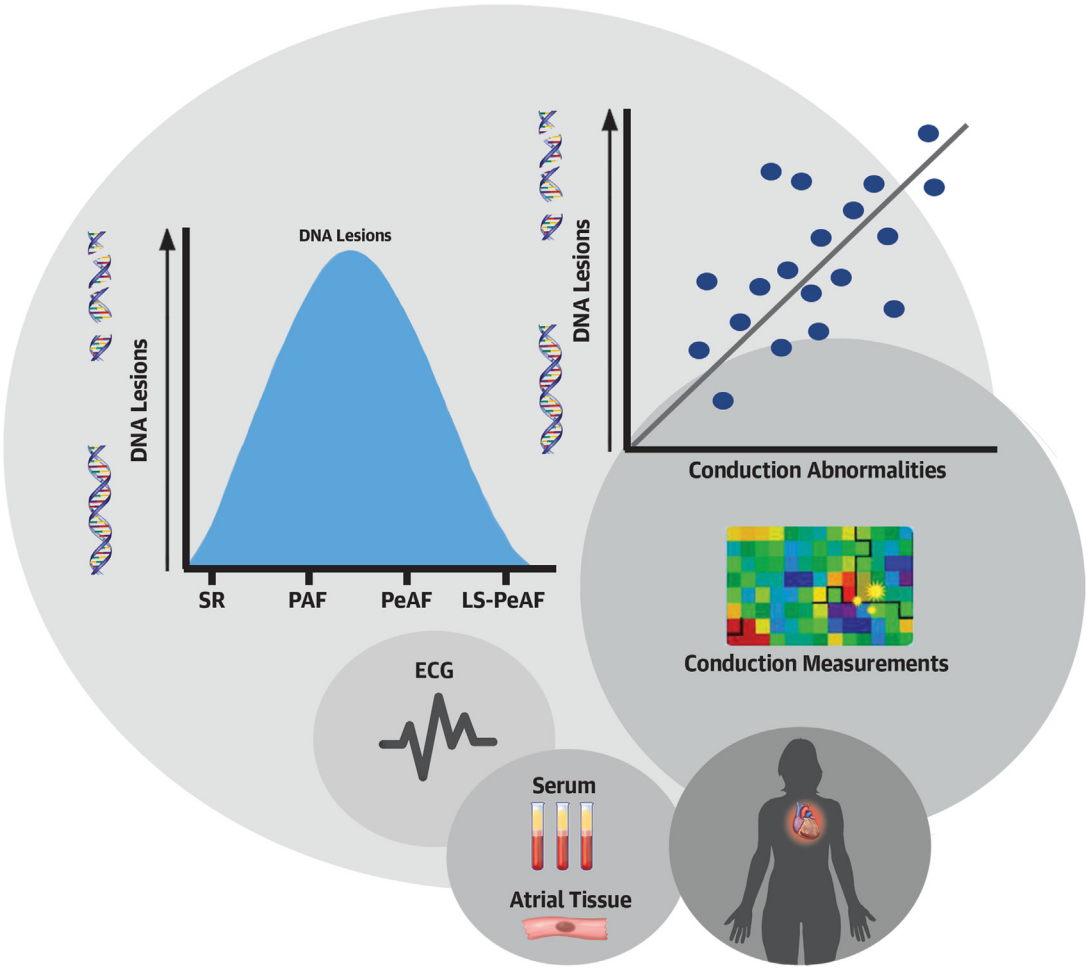
NUMBER OF DNA LESIONS IS ASSOCIATED WITH ELECTRICAL CONDUCTION ABNORMALITIES. An important observation is that the number of DNA lesions correlates with the proportion of electrical conduction abnormalities, such as a gradual decrease in conduction velocity and the presence of fractionated potentials. Electrical conduction abnormalities correlate with nDNA lesions in SR patients and with mtDNA lesions in AF patients, indicating that nDNA lesions are associated with conduction abnormalities independently from the presence of previous AF episodes. On the other hand, mtDNA lesions may be a result of AF, which is in line with experimental and clinical studies showing a key role for mitochondrial dysfunction in AF promotion.[7,26,27]

Previous mapping studies demonstrated that even during SR, patients with a history AF have more conduction abnormalities than patients without atrial tachyarrhythmias.[28,29] Conduction abnormalities are a key element of the arrhythmogenic substrate underlying AF. Patients with LS-PeAF have extensive conduction abnormalities along the lateral boundaries of the atrial musculature, resulting in a more than 6-fold higher incidence of intra-atrial conduction block compared with patients with acutely induced AF.[2,3] These multiple lines of intra-atrial conduction block were associated with an increase in the number of fibrillation waves, thereby increasing the complexity of the fibrillatory process. Low potential voltages and fractionation are indicators of conduction abnormalities.[30] Because the amount of low potential voltages and fractionation are associated with the number of DNA lesions, quantification of DNA lesions may therefore represent a substitute to determine the amount of conduction disorders, which could aid in the staging of AF.

THE CONCENTRATION OF SERUM DNA FOR AF DIAGNOSTICS. The levels of serum cfDNA, especially β-globin and P53 concentrations, are prognostic and predictive markers in patients with cancer.[31-34] To date, it is not known whether serum cfDNA levels could be used as diagnostic marker to stage the severity of AF. In the present study, the concentration of cfDNA was not associated with the classic clinical stages of AF, which is in agreement with a study by Wiersma et al.[7] Nevertheless, cfDNA levels were strongly related to the time elapsed since AF diagnosis and discriminate between short- and long-term AF, particularly for the mitochondrial marker. Previous studies have reported on sustained mitochondrial damage as drivers of mitochondrial dysfunction[35,36] that are associated with initial increase and ultimately exhaustion of cfDNA levels over time.[7,26] This may explain the lower cfDNA levels observed in patients with long-term AF. Generally, most serum DNA levels in tissue and blood are analyzed with the quantitative PCR approach, which

JACC: CLINICAL ELECTROPHYSIOLOGY VOL. 11, NO. 2, 2025
FEBRUARY 2025:321–332

CENTRAL ILLUSTRATION The Quantity of DNA Lesions Is Associated With Atrial Conduction Abnormalities and Stage of AF



Pool L, et al. JACC Clin Electrophysiol. 2025;11(2):321-332.

ECG = electrocardiography; LS-PeAF = longstanding persistent atrial fibrillation; PAF = paroxysmal atrial fibrillation; PeAF = persistent atrial fibrillation; SR = sinus rhythm.

is primer-specific and template-specific, labor intensive, and therefore cost-inefficient. In the present study, we found a close to perfect correlation between cfDNA concentrations obtained with the use of PicoGreen assay and serum DNA concentrations of P53 and β-globin. Therefore, the PicoGreen assay can be used as an easy, efficient, and robust approach to quantify cfDNA levels in serum samples.

DIFFERENCES BETWEEN nDNA AND mtDNA. In this study, we show superior association between the number of mtDNA lesions with AF staging compared with the number of nDNA lesions. This emphasizes the notion of a key role for mitochondrial function and energy demand during the high activation rate of atrial cardiomyocytes during AF.[26] AF-related mitochondrial dysfunction is linked to the release of reactive oxygen species (ROS), which in turn increases oxidative DNA damage and excessive activation of the DNA repair protein poly(ADP)-ribose polymerase 1 (PARP1).[4] Activated PARP1 synthesis ADP-ribose chains, and in turn depletes mitochondrial

nicotinamide adenine dinucleotide levels, resulting in further DNA damage and contractile dysfunction of atrial cardiomyocytes. This feed-forward mechanism, driven by mitochondrial ROS production, may explain the increase in mitochondrial serum DNA levels in AF. In line with this, it has been reported that mtDNA damage is more persistent and susceptible to damage by ROS compared with nDNA owing to the close proximity of the electron transport chain, the major source of ROS production, and lack of compaction around histones.[37,38] Cardiomyocytes are known to degrade dysfunctional mitochondria via mitophagy,[39] which may explain the reduced number of mtDNA lesions in long-term vs short-term AF patients (Supplemental Figure 8).

STUDY LIMITATIONS. Patients with various underlying heart diseases were included in this study and therefore the AF pathologies maybe differ. Although multivariate analyses did not show significant correlations between underlying heart diseases and the quantity of DNA damage, further research is warranted to elucidate its impact. The measurement of DNA damage as a marker to stage AF may aid in the selection of AF stage-tailored therapy and consequently improve the effectiveness of the therapy. To establish this, larger-scale prospective trials are warranted.

CONCLUSIONS

The quantity of DNA lesions in atrial tissue samples reflects the clinical stage of AF, time since AF diagnosis, and the proportion of atrial conduction disorders as measured during SR (**Central Illustration**). Furthermore, mitochondrial serum DNA damage markers discriminate short- from long-term AF. This observation indicates that the quantity of DNA damage and serum DNA levels have a potential diagnostic value to predict the amount of electropathology and clinical stage of atrial fibrillation in the individual patient.

DATA AVAILABILITY

The data underlying this paper will be shared on reasonable request to the corresponding author.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This research was funded by Atrial-Fibrillation-Innovation-Platform (AFIPonline.org), Dutch Heart Foundation (DnAFix 2020-2020B003), NWO-Vidi (2016-91717339 to Dr de Groot), and Medical Delta NWA-ORC project CIRCULAR NWO (NWA.1389.20.157). The authors have no relationships relevant to the contents of this paper to disclose.

ADDRESS FOR CORRESPONDENCE: Prof Dr Bianca J.J.M. Brundel, Department of Physiology, Amsterdam University Medical Centers, location VUmc, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands. E-mail: b.brundel@amsterdamumc.nl.

PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: The quantity of DNA lesions in atrial tissue samples reflect the clinical stage of AF, time since AF diagnosis, and the proportion of atrial conduction disorders as measured during SR. Furthermore, mitochondrial serum DNA damage markers discriminate short- from long-term AF.

TRANSLATIONAL OUTLOOK: Future studies should indicate whether the quantity of DNA damage and serum DNA levels have a potential diagnostic value to predict the amount of electropathology and clinical stage of atrial fibrillation in the individual patient.

REFERENCES

1. Brundel BJJM, Ai X, True Hills M, et al. Atrial fibrillation. *Nat Rev Dis Primers*. 2022/04/07;8(1):21, 2022.

2. de Groot NM, Houben RP, Smeets JL, et al. Electropathological substrate of longstanding persistent atrial fibrillation in patients with structural heart disease: epicardial breakthrough. *Circulation*. 2010;122(17):1674–1682.

3. Allessie MA, de Groot NMS, Houben RP, et al. Electropathological substrate of long-standing persistent atrial fibrillation in patients with structural heart disease: longitudinal dissociation. *Circ Arrhythm Electrophysiol*. 2010;3(6):606–615.

4. Zhang D, Hu X, Li J, et al. DNA damage-induced PARP1 activation confers cardiomyocyte dysfunction through NAD^+ depletion in experimental atrial fibrillation. *Nat Commun*. 2019;10(1):1307.

5. Ramos KS, Brundel BJJM. DNA damage, an innocent bystander in atrial fibrillation and other cardiovascular diseases? *Front Cardiovasc Med*. 2020;7:67.

6. Li J, Zhang D, Ramos KS, et al. Blood-based 8-hydroxy-2′-deoxyguanosine level: a potential diagnostic biomarker for atrial fibrillation. *Heart Rhythm*. 2021;18(2):271–277.

7. Wiersma M, van Marion DMS, Bouman EJ, et al. Cell-free circulating mitochondrial DNA: a potential blood-based marker for atrial fibrillation. *Cells*. 2020;9(5):1159.

8. Lehle S, Hildebrand DG, Merz B, et al. LORD-Q: a long-run real-time PCR-based DNA-damage quantification method for nuclear and mitochondrial genome analysis. *Nucleic Acids Res*. 2014;42(6). e41-e41.

9. Santos JH, Meyer JN, Mandavilli BS, van Houten B. Quantitative PCR-based measurement of nuclear and mitochondrial DNA damage and repair in mammalian cells. *DNA Repair Protoc Mamm Syst*. 2006:183–199.

10. Gonzalez-Hunt CP, Rooney JP, Ryde IT, et al. PCR-based analysis of mitochondrial DNA copy number, mitochondrial DNA damage, and nuclear DNA damage. *Curr Protoc Toxicol*. 2016;67(1):20.11.1–25.

11. Khan K, Tewari S, Rastogi M, et al. Quantitative extra long PCR to detect DNA lesions in patients exposed to low doses of diagnostic radiation. *Asian Pac J Cancer Prev*. 2018;19(5):1367.

12. Hindricks G, Potpara T, Dagres N, et al. 2020 ESC guidelines for the diagnosis and management of atrial fibrillation developed in collaboration with the European Association for Cardio-Thoracic Surgery (EACTS): the Task Force for the Diagnosis and Management of Atrial Fibrillation of the European Society of Cardiology (ESC) developed with the special contribution of the European Heart Rhythm Association (EHRA) of the ESC. *Eur Heart J*. 2021;42(5):373–498.

13. Siejka-Zielinska P, Cheng J, Liu Y, et al. Cell-free DNA TAPS provides multimodal information for early cancer detection. *Sci Adv*. 2021;7(36):eabh0534.

14. Shin SH, Park WY, Park D. Characterization of DNA lesions associated with cell-free DNA by targeted deep sequencing. *BMC Med Genomics*. 2021;14(1):192.

15. Zhou Z, Ma MJL, Chan RWY, et al. Fragmentation landscape of cell-free DNA revealed by deconvolutional analysis of end motifs. *Proc Natl Acad Sci U S A*. 2023;120(17):e2220982120.

16. Kottkamp H, Tanner H, Kobz R, et al. Time courses and quantitative analysis of atrial fibrillation episode number and duration after circular plus linear left atrial lesions: trigger elimination or substrate modification: early or delayed cure? *J Am Coll Cardiol*. 2004;44(4):869–877.

17. de Groot N, van der Does L, Yaksh A, et al. Direct proof of endo-epicardial asynchrony of the atrial wall during atrial fibrillation in humans. *Circ Arrhythm Electrophysiol*. 2016;9(5):e003648.

18. Thijssen VLJL, Ausma J, Liu GS, Allessie MA, van Eys GJ, Borgers M. Structural changes of atrial myocardium during chronic atrial fibrillation. *Cardiovasc Pathol*. 2000;9(1):17–28.

19. Dos Santos Á, Cook AW, Gough RE, et al. DNA damage alters nuclear mechanics through chromatin reorganization. *Nucl Acids Res*. 2021;49(1):340–353.

20. Ausma J, Wijffels M, Thoné F, Wouters L, Allessie M, Borgers M. Structural changes of atrial myocardium due to sustained atrial fibrillation in the goat. *Circulation*. 1997;96(9):3157–3163.

21. Ausma J, Borgers M. Dedifferentiation of atrial cardiomyocytes: from in vivo to in vitro. *Cardiovasc Res*. 2002;55(1):9–12.

22. Thijssen VL, Ausma J, Borgers M. Structural remodelling during chronic atrial fibrillation: act of programmed cell survival. *Cardiovasc Res*. 2001;52(1):14–24.

23. Puspitasari A, Cerri M, Takahashi A, Yoshida Y, Hanamura K, Tinganelli W. Hibernation as a tool for radiation protection in space exploration. *Life*. 2021;11(1):54.

24. Brundel BJJM, Ausma J, van Gelder IC, et al. Activation of proteolysis by calpains and structural changes in human paroxysmal and persistent atrial fibrillation. *Cardiovasc Res*. 2002;54(2):380–389.

25. Thijssen VLJL, Ausma J, Gorza L, et al. Troponin I isoform expression in human and experimental atrial fibrillation. *Circulation*. 2004;110(7):770–775.

26. Wiersma M, van Marion DMS, Wüst RCI, et al. Mitochondrial dysfunction underlies cardiomyocyte remodeling in experimental and clinical atrial fibrillation. *Cells*. 2019;8(10):1202.

27. Li J, Qi X, Ramos KS, et al. Disruption of sarcoplasmic reticulum–mitochondrial contacts underlies contractile dysfunction in experimental and human atrial fibrillation: a key role of mitofusin 2. *J Am Heart Assoc*. 2022;11(19):e024478.

28. Heida A, van der Does WFB, van Staveren LN, et al. Conduction heterogeneity: impact of underlying heart disease and atrial fibrillation. *JACC Clin Electrophysiol*. 2020;6(14):1844–1854.

29. van Schie MS, Veen D, Kharbanda RK, et al. Characterization of pre-existing arrhythmogenic substrate associated with de novo early and late postoperative atrial fibrillation. *Int J Cardiol*. 2022;363:71–79.

30. van Schie MS, Starreveld R, Roos-Serote MC, et al. Classification of sinus rhythm single potential morphology in patients with mitral valve disease. *Europace*. 2020;22(10):1509–1519.

31. Fernandez-Garcia D, Hills A, Page K, et al. Plasma cell-free DNA (cfDNA) as a predictive and prognostic marker in patients with metastatic breast cancer. *Breast Cancer Res*. 2019;21(1):149.

32. Arshad S, Khawar MB, Hassan A, et al. Cell free DNA; diagnostic and prognostic approaches to oncology. *Adv Cancer Biol Metastasis*. 2022;5:100052.

33. Lo YMD, Tein MSC, Lau TK, et al. Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am J Hum Genet*. 1998;62(4):768–775.

34. Mattioni M, Soddu S, Prodosmo A, et al. Prognostic role of serum p53 antibodies in lung cancer. *BMC Cancer*. 2015;15:148.

35. Montaigne D, Marechal X, Lefebvre P, et al. Mitochondrial dysfunction as an arrhythmogenic substrate: a translational proof-of-concept study in patients with metabolic syndrome in whom post-operative atrial fibrillation develops. *J Am Coll Cardiol*. 2013;62(16):1466–1473.

36. Kim YM, Guzik TJ, Zhang YH, et al. A myocardial Nox2 containing NAD(P)H oxidase contributes to oxidative stress in human atrial fibrillation. *Circ Res*. 2005;97(7):629–636.

37. Yakes FM, van Houten B. Mitochondrial DNA damage is more extensive and persists longer than nuclear DNA damage in human cells following oxidative stress. *Proc Natl Acad Sci U S A*. 1997;94(2):514–519.

38. Santos RX, Correia SC, Zhu X, et al. Mitochondrial DNA oxidative damage and repair in aging and Alzheimer's disease. *Antioxid Redox Signal*. 2013;18(18):2444–2457.

39. Jimenez RE, Kubli DA, Gustafsson ÅB. Autophagy and mitophagy in the myocardium: therapeutic potential and concerns. *Br J Pharmacol*. 2014;171(8):1907–1916.

KEY WORDS **atrial fibrillation, circulating free DNA, diagnostics, DNA damage, electrical conduction abnormalities, serum**

APPENDIX **For supplemental Methods, tables, and figures, please see the online version of this paper.**