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Hemolysis after pulsed-field ablation in pulmonary vein isolation for atrial fibrillation: A prospective controlled trial

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ABSTRACT

BACKGROUND Intravascular hemolysis with consecutive acute kidney injury (AKI) has been described after pulsed-field ablation (PFA) in atrial fibrillation (AF).

OBJECTIVE This study aimed to evaluate the risk of intravascular hemolysis and AKI after PFA with different numbers and forms of applications.

METHODS From May 2022 to August 2024, consecutive patients undergoing AF ablation were prospectively enrolled in 4 groups, including equally distributed numbers of patients undergoing PFA (pentaspline catheter) with 8 applications per vein (PFA-8), with 16 applications per vein (PFA-16), CardioFocus focal PFA, and radiofrequency ablation (RFA). Blood samples were collected immediately before and after the ablation, and on the following day to analyze hemolysis markers and indicators for AKI.

RESULTS A total of 200 patients (68.4 ± 10.8 years) could be included in the final analysis. The blood samples revealed a significant increase in total bilirubin and lactate dehydrogenase across all PFA modalities and RFA 24 hours after ablation compared with baseline levels. Potassium levels increased significantly in all PFA modalities immediately post-ablation as compared with baseline, followed by a return to approximately baseline after 24 hours. No significant potassium fluctuations were observed in RFA. Serum creatinine levels showed no significant increase in any PFA modality or RFA within the 24-hour assessment period.

CONCLUSION PFA using a multispline catheter with 8 and 16 applications per PV and focal PFA using CardioFocus (CardioFocus Inc., Massachusetts) platform showed no safety concerns with respect to hemolysis-induced AKI.

KEYWORDS Atrial Fibrillation; Pulmonary Vein Isolation; Catheter Ablation; Safety; Pulsed Field Ablation; CardioFocus focal pulsed field ablation; Radiofrequency Ablation; Acute kidney injury; Hemolysis

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Introduction

Pulmonary vein isolation (PVI) has become an increasingly significant method for the treatment of atrial fibrillation (AF).[1] Thermal modalities of PVI, such as radiofrequency ablation (RFA) or cryoballoon ablation may cause damage to surrounding tissues of the pulmonary veins (PVs).[2] In rare cases, these procedures may result in esophageal damage or, in severe instances, a potentially lethal atrio-esophageal fistula.[3,4] Pulsed-field ablation (PFA) has recently emerged as a nonthermal energy source for PVI with the aim to reduce the effect on surrounding tissues by selectively targeting myocardial tissue.[5–7] However, severe acute kidney injury (AKI) related to hemolysis has been described as a possible complication of PFA.[8,9]

As PFA is a relatively new method for AF ablation, data comparing the available PFA systems to determine the optimal number of pulses based on a risk-benefit analysis remains limited. Furthermore, studies comparing PFA with RFA

regarding feasibility, effectiveness, and safety are still pending.

The objective of this study was to assess the risk of intravascular hemolysis and the incidence of AKI across different types and numbers of applications of PFA compared with RFA.

Methods

From May 2022 to August 2024, a total of 200 consecutive patients (68.4 ± 10.8 years) undergoing AF ablation were prospectively enrolled. The study protocol was approved by the local ethics committee of Heinrich Heine University Düsseldorf (Study Number 2019-555_4) and complies with the Declaration of Helsinki as revised in 2013. Informed written consent was obtained from all study participants.

Study population and quasi-randomization

A quasi-randomization ensures the equal distribution of patients across the 4 ablation groups (PFA-8, PFA-16, PFA-foc, and RFA) by employing a systematic scheduling approach rather than random allocation.

All patients were assigned to the different ablation modalities based on the order of accessible time slots, which were pre-determined for each PVI modality in an alternating order. This approach minimizes potential biases by ensuring that the treating electrophysiologists had no influence over the choice of ablation type, the timing, or the assignment of specific time slots for each patient. A similar quasi-randomization method has been described before.[10]

Ablation procedures

Oral anticoagulation was maintained on the day of the procedure in all patients. Experienced electrophysiologists performed each ablation under conscious sedation and analgesia using an initial bolus of midazolam, followed by continuous propofol infusion with continuous monitoring of blood pressure, heart rate, and oxygen saturation. In all cases, intra-cardiac thrombi could be ruled out by transesophageal echocardiography. None of the patients was intubated.

The femoral venous puncture was performed with ultrasound guidance. A transseptal puncture was performed under transesophageal echocardiographic and fluoroscopic guidance with a steerable sheath. Intravenous heparin was administered to achieve an activated clotting time above 300 seconds throughout the procedure. At the end of the procedure, all PVs were checked for entrance and exit block.

Abbreviations

AF: atrial fibrillation

AKI: acute kidney injury

eGFR: estimated glomerular filtration rate

LDH: lactate dehydrogenase

PFA: pulsed-field ablation

PFA-16: pulsed-field ablation with 16 applications per vein

PFA-8: pulsed-field ablation with 8 applications per vein

PFA-foc: CardioFocus focal pulsed-field ablation

PVI: pulmonary vein isolation

RFA: radiofrequency ablation

PFA with the pentaspline catheter (PFA-8 and PFA-16)

Our workflow for PFA using a pentaspline catheter has been described previously.[11] In summary, a direct transseptal puncture was performed under both echocardiographic and fluoroscopic guidance with the FARADRIVE Steerable Sheath (FARADRIVE, Boston Scientific Inc., Marlborough, Massachusetts). The pentaspline PFA catheter (FARAWAVE, Boston Scientific Inc.) was then advanced through the FARADRIVE sheath and positioned in the left atrium (LA). Subsequent steps and the ablation procedure adhered to the manufacturer's guidelines.

Each PFA application consisted of 5 bursts of bipolar PFA impulses delivered through the pentaspline PFA catheter over 2500 ms, with each burst applied at intervals of 500 ms. A standard J-wire was used to intubate each PV, with 4 PFA applications administered in the "basket" configuration and another 4 PFA applications in the "flower" configuration (PFA-8).[12] After every 2 applications per configuration, the catheter was manually rotated. In the PFA-16 group, each PV received 6 applications in the "basket" configuration and 10 in the "flower" configuration.

Tissue contact with the catheter was assessed fluoroscopically by observing characteristic changes in catheter configuration.

The ablation was performed in the following order: Left superior PV, left inferior PV, right superior PV, and right inferior PV.

CardioFocus focal PFA

The CardioFocus focal PFA (PFA-foc) was performed with the transseptal sheath and 3D mapping system as employed for RFA. In contrast to RFA, a THERMOCOOL SMARTTOUCH catheter (Biosense Webster Inc., California) was connected to the CENTAURI generator for PFA (CardioFocus Inc., Massachusetts). Each ablation point consisted of a 25 Ampere application, consisting of 10 bursts of unipolar impulses delivered over a period of 10 seconds.

RFA

Our standardized ablation protocol for RFA was a modified CLOSE protocol[13]: The anterior segment of the PV was ablated using 50W (high power short duration) and a target ablation index of 550, whereas the posterior segment was treated with 90W for 4 seconds (very high power short duration).

For transseptal access during RFA procedures, the Agilis NxT steerable sheath (Abbott, Illinois) was used. In all cases, a 3D mapping system (CARTO, Biosense Webster Inc., California) was used with a QDOT Micro catheter (Biosense Webster Inc., California), aiming for a contact force of 10 to 20 g.

Biological analysis

Blood samples in serum probes were taken immediately before and after the PVI, directly from the coronary sinus at the beginning (T0) and at the end of the procedure (T1). A third blood sample was taken from a peripheral vein (T2) after 24 hours. From these blood samples, we evaluated the hemolysis markers total bilirubin, lactate dehydrogenase (LDH),

potassium, and the markers for AKI such as creatinine, urea, and the estimated glomerular filtration rate (eGFR).

Bilirubin

Total bilirubin was analyzed with the DPD method using the clinical chemistry module cobas 8000, c502 (Roche Diagnostics GmbH, Baden-Württemberg, Germany). A hyperbilirubinemia was assumed at a value of >1 mg/dl.

LDH

LDH was analyzed to evaluate the extent of hemolysis. It was photometrically determined using the clinical chemistry module cobas 8000, c701 (Roche Diagnostics GmbH).

Potassium

Potassium was analyzed using an ion-selective electrode to convert the effective concentration of the ion dissolved in solution into an electric potential. The electric potential was measured by a voltmeter. This analysis method is according to common procedures.[14]

Creatinine

Creatinine was evaluated from a serum probe in a photometric assay test with an enzymatic cycling method that uses creatinine kinase[15] in the clinical chemistry module cobas 8000, c701 (Roche Diagnostics GmbH).

Urea

Urea was analyzed from a serum probe to evaluate the kidney function using the chemistry module cobas 8000, c502 (Roche Diagnostics GmbH).

eGFR

The eGFR was calculated using the chronic kidney disease-epidemiology collaboration equation.[16]

Statistical analysis

Continuous variables are depicted as the arithmetic mean with standard deviation or median with interquartile range. They were compared using the Student t test.
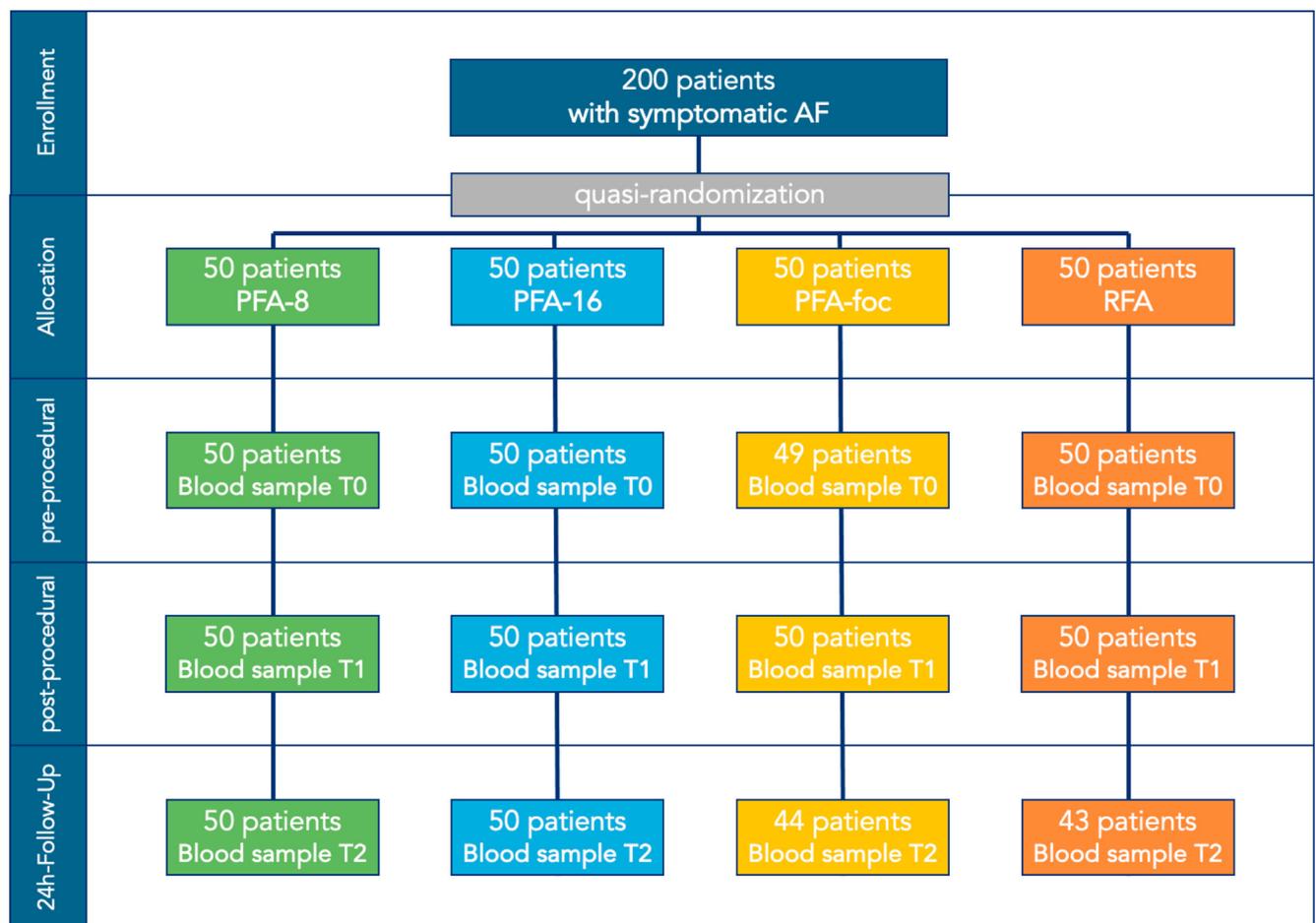

Figure 1
Overview. A total of 200 patients underwent a quasi-randomization into the following 4 groups of ablation types: PFA-8, PFA-16, PFA-foc, and RFA. In each group, a blood sample was taken at the beginning (T0), at the end of the procedure (T1), and on the day after the procedure (T2). All enrolled patients underwent the procedure and were included in the statistical analysis. AF = atrial fibrillation; PFA = pulsed-field ablation; PFA-foc = CardioFocus focal PFA; RFA = radiofrequency ablation.

Table 1 Baseline characteristics I

Patient characteristics	All patients (n = 200)	PFA-8 (n = 50)	PFA-16 (n = 50)	PFA-foc (n = 50)	RFA (n = 50)	P value
Age, yrs	67 (59–76)	67 (58–73)	64 (59–78)	67 (59–75)	70 (63–78)	0.39
Men, n (%)	107 (53%)	25 (50)	25 (50)	27 (24)	30 (60)	0.71
Height (cm)	174 (168–182)	174 (168–180)	175 (168–183)	174 (165–185)	174 (170–182)	0.72
Weight (kg)	85 (70–99)	88 (71–96)	84.5 (70–100)	87.5 (72–103)	80 (70–94)	0.70
BMI (kg/m^2)	27 (24–32)	27 (24–31)	27 (24–32)	27 (24–34)	26 (24–30)	0.58
CHA$_2$DS$_2$-VASc score	3 (1–4)	3 (2–4)	2 (1–3)	3 (1–4)	3 (2–4)	0.38
AF type – n (%)	200 (100%)	50 (100%)	50 (100%)	50 (100%)	50 (100%)	–
- paroxysmal (%)	131 (65)	33 (66)	39 (78)	31 (62)	28 (56)	0.42
- persistent (%)	64 (32)	16 (32)	10 (20)	19 (38)	19 (38)	0.55
- long-standing persistent (%)	5 (3)	1 (2)	1 (2)	0 (0)	2 (6)	0.33

This table presents a summary of the patient characteristics and the relevant medical history of the specific groups and all patients in total.
AF = atrial fibrillation; BMI = body mass index.

Categorial variables are reported as numbers and percentages. They were respectively compared using the χ^2 or the Fisher's exact test.

The independent Kruskal-Wallis Test was used as a nonparametric method for testing whether the blood samples of the 4 patient groups originate from the same distribution.

A P-value < .05 was set as statistically significant. For data processing and analysis, SPSS (IBM SPSS Statistics, Version 29.0.2.0 (20), New York) was used.

A power analysis was conducted to determine the required sample size for this study.

Results

A total of 200 patients underwent a quasi-randomization into the 4 groups of ablation types which were PFA-8, PFA-16, PFA-foc, and RFA. In each group, a blood sample was taken at the beginning (T0) and at the end of the procedure (T1), and on the day after the procedure (T2). All enrolled patients underwent the procedure and were included in the statistical analysis (Figure 1).

Patient characteristics

The patient characteristics of the study population are summarized in Table 1 and Table 2. Overall, 200 patients suffering from symptomatic AF underwent ablation. The mean patient age was 68.37 ± 10.78 years. A total of 132 patients (66%) were male. The EHRA score was at least 2 and the median CHA$_2$DS$_2$-VASc score was 2.78 ± 1.58.

Regarding patient characteristics, including pre-existing conditions, and previous cardiac interventions or surgeries, no significant difference was found when comparing the 4 groups of ablation types (Table 1 and Table 2).

Ablation procedure

All PVs were isolated. The procedural data are summarized in Table 3.

Significant differences were found in terms of the procedural duration, the time to transseptal puncture, and the fluoroscopy time with the lowest procedural duration and time to transseptal puncture in PFA-16 and PFA-8 and similar data comparing PFA-foc and RFA. The lowest fluoroscopy time was observed in PFA-foc, followed by PFA-8, RFA, and PFA-16.

No significant difference was observed in the fluoroscopy dose between the groups (Table 3).

Increase of hemolysis parameters after ablation

In the following, the blood results of the investigated parameters of hemolysis are compared between the 4 ablation types: PFA-8, PFA-16, PFA-foc, and RFA.

Table 2 Baseline characteristics II

Comorbidities	All patients (n = 200)	PFA-8 (n = 50)	PFA-16 (n = 50)	PFA-foc (n = 50)	RFA (n = 50)	P value
Heart failure – n (%)	23 (12)	6 (12)	7 (14)	4 (8)	6 (12)	0.83
Arterial hypertension – n (%)	127 (63)	34 (68)	26 (52)	35 (70)	32 (64)	0.24
Diabetes mellitus – n (%)	28 (14)	6 (12)	6 (12)	10 (20)	6 (12)	0.57
Coronary artery disease – n (%)	54 (27)	11 (22)	16 (32)	13 (26)	14 (28)	0.72
Coronary artery bypass graft – n (%)	4 (2)	2 (4)	0 (0)	1 (2)	1 (2)	0.56
History of MI – n (%)	23 (12)	5 (10)	6 (12)	8 (16)	4 (8)	0.63
Valvular surgery – n (%)	11 (6)	2 (4)	0 (0)	4 (8)	5 (10)	0.12
Valvular intervention – n (%)	2 (1)	2 (4)	0 (0)	0 (0)	0 (0)	0.10
History of stroke/TIA – n (%)	10 (5)	3 (6)	2 (4)	2 (4)	3 (6)	0.93
Obstructive sleep apnea – n (%)	29 (14)	8 (16)	6 (12)	7 (14)	8 (16)	0.93

This table presents a summary of the patient characteristics and the relevant medical history of the specific groups and all patients in total.
LVEF = left ventricular ejection fraction; MI = myocardial infarction; TIA = transient ischemic attack.

Table 3 Procedural characteristics

Procedural data	All patients (n = 200)	PFA-8 (n = 50)	PFA-16 (n = 50)	PFA-foc (n = 50)	RFA (n = 50)	P value
Procedural duration – min	74 (48–123)	45 (36–50)	55 (48–68)	123 (103–136)	124 (101–150)	0.001
Total number of applications	N/A	32	64	67.41 (4.31)	66.58 (4.54)	N/A
Time to TSP – min	16 (14–21)	15 (12–17)	15 (14–20)	20 (14–25)	19 (13–25)	0.006
Fluoroscopy time – min	13 (9–18)	12 (9–14)	17 (14–23)	10 (6–17)	15 (9–19)	0.001
Fluoroscopy dose – cGy cm^2	496 (361–754)	434 (344–682)	553 (404–910)	392 (321–628)	604 (436–831)	0.076

The relevant data of the ablation procedure is shown in this table.
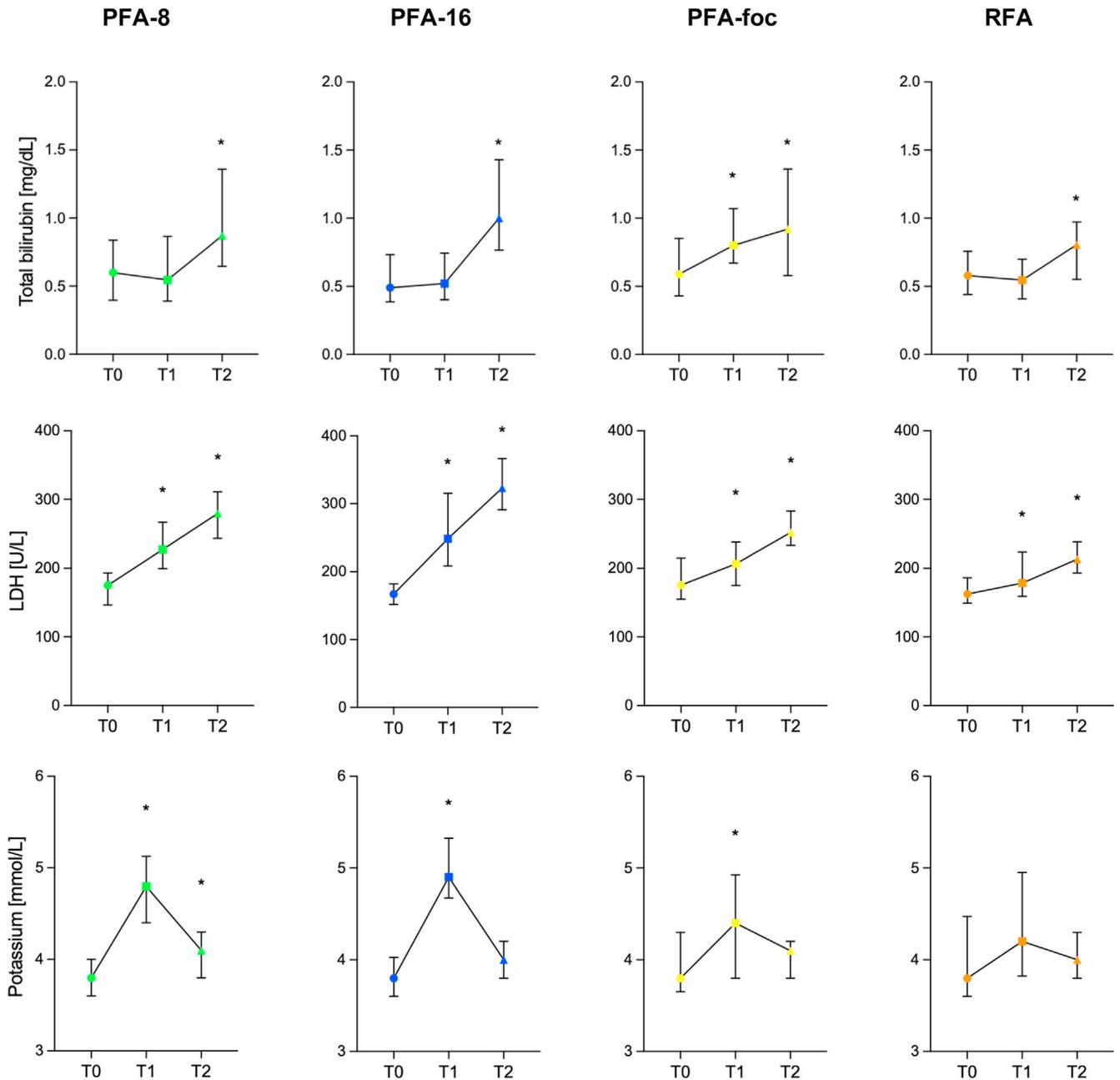TSP = transseptal puncture.

Figure 2

Hemolysis parameters compared across the 4 ablation types. The concentration of total bilirubin, LDH, and potassium at the 3 time points of blood samples (pre-procedural (T0), post-procedural (T1), and 1 day after the procedure (T2) are compared between the 4 ablation groups shown as an arithmetic mean. Potassium levels increased significantly in all PFA modalities immediately post-ablation as compared to baseline, followed by a return to approximately baseline after 24 hours. No significant potassium fluctuations were observed in RFA.
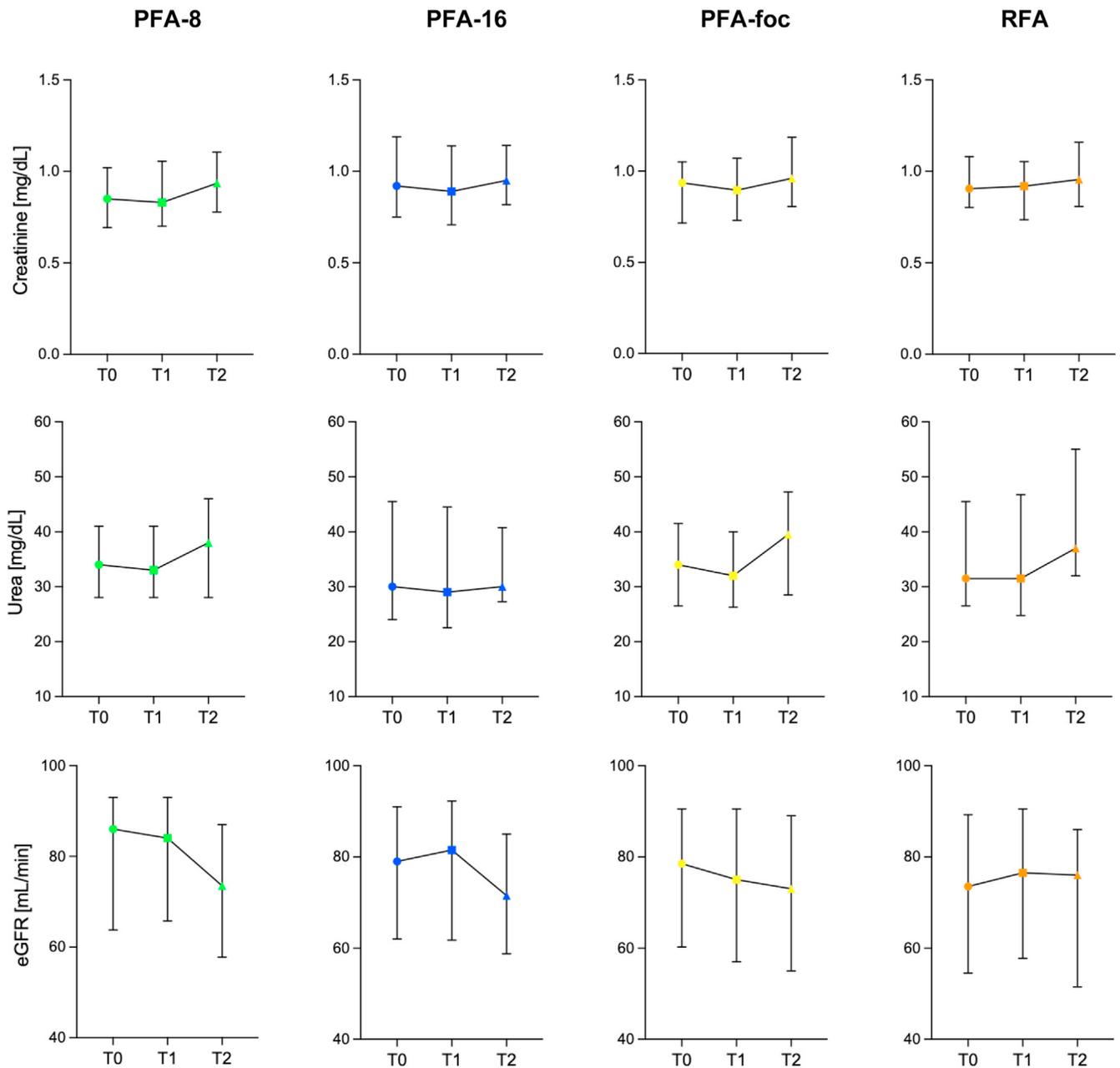
Figure 3
Kidney function compared across the 4 ablation types. The concentration of serum creatinine, serum urea, and estimated glomerular filtration rate (eGFR) at the following 3 time points of blood samples: pre-procedural (T0), post-procedural (T1), and 1 day after the procedure (T2) are compared among the 4 ablation groups shown as an arithmetic mean with no significant dynamic.

A significant increase in total bilirubin and LDH after ablation was found for all groups (Figure 2).

Kidney function after ablation

In addition to the hemolysis parameters, the blood results of the investigated parameters of AKI are compared among the following 4 types of ablations: PFA-8, PFA-16, PFA-foc, and RFA.

No significant increase in any of these parameters was found after ablation for any group (Figure 3).

Complications

Major complications

In 1% of the patients (1 patient undergoing PFA-16), a pericardial tamponade related to the insertion of a coronary sinus diagnostic catheter, and a third-degree atrioventricular block after

Table 4 Complications

	All patients (n = 200)	PFA-8 (n = 50)	PFA-16 (n = 50)	PFA-foc (n = 50)	RFA (n = 50)
Major complications:					
Pericardial tamponade n (%)	1 (0.5%)	0 (0%)	1 (2.0%)	0 (0%)	0 (0%)
Permanent gastroparesis n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Stroke n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Transient ischemic attack n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Atrio-esophageal fistula n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Third-degree atrioventricular block n (%)	0 (0%)	1 (2.0%)	0 (0%)	0 (0%)	0 (0%)
Death n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Minor complications:					
Groin complications n (%)	2 (1.0%)	0 (0%)	1 (2.0%)	1 (2.0%)	0 (0%)
Pericardial effusion n (%)	1 (0.5%)	0 (0%)	0 (0%)	0 (0%)	1 (2.0%)
Pneumonia n (%)	1 (0.5%)	0 (0%)	0 (0%)	0 (0%)	1 (2.0%)
Transient gastroparesis n (%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

electro-cardioversion (1 patient undergoing PFA-8) occurred. No other major complications were reported (Table 4).

Minor complications

In 2% of the cases, we documented groin complications that were successfully treated at our department of cardiology and vascular medicine (1 patient undergoing PFA-16, 1 patient undergoing PFA-foc). In 0.5% of the cases, pericardial effusion (1 patient undergoing RFA) and pneumonia (1 patient undergoing RFA) were reported (Table 4).

Discussion

Multiple factors may contribute to PFA-related hemolysis, including catheter characteristics, ablation metrics, mode of energy delivery, and tissue contact.

The correlation between the number of PFA applications and the occurrence of hemolysis and AKI remains insufficiently investigated. In addition, there is still limited data regarding the optimal number of PFA applications for long-term freedom from AF.

Especially a comparative investigation of PFA with different applications per vein as shown in our study (8 as recommended by the manufacturer vs 16 as double the recommended number) and the comparison of this bipolar PFA with unipolar PFA-foc in terms of safety has not been sufficiently addressed in previous studies.

Ekanem and coworkers[8] demonstrated that up to 100 PFA applications, including ablation of the posterior wall, can be considered safe in the interventional treatment of AF. We hypothesize that 16 applications per PV may be more effective than 8. Therefore, our study protocol deviates from the manufacturer's standard recommendation of 8 applications per PV. The follow-up is ongoing, and results will be published in a separate study comparing the effectiveness in terms of the number of applications.

While the increase of bilirubin, LDH, and potassium indicates hemolysis consecutive to the ablation, the absence of AKI after ablation in all groups can be seen as an indicator of the safety of all investigated ablation types. Other potential side effects of hemolysis, besides AKI, have not yet been described.

Previous studies suggest that hemolysis may occur during PFA, with the risk increasing as the number of PFA applications rises.[17,18]

Ekanem and coworkers[8] demonstrated that hemolysis-related AKI occurred in 5 out of 17.000 patients. In these cases, between 96–159 PFA applications were delivered with a mean of 143 ($\pm$ 27) applications (96, 144, 158, 159, and 159).

To further investigate the correlation between PFA energy applications and hemolysis, an in vitro study in mice demonstrated hemolysis at PFA intensities of 1,000 V/m, whereas 75% of cardiomyocyte death was observed at 1,500 V/m.[19] Mohanty and coworkers[20] suggest that high-voltage PFA leads to the accumulation of hem in renal glomeruli, resulting in AKI.

To summarize, our main findings are in line with other studies concerning hemolysis after ablation.

Our study has certain limitations. We cannot rule out a delayed increase in creatinine after 48 hours, as the final blood sample was taken at 24 hours, which is standard in similar studies (eg, Osmancik and coworkers, 2024; Mohanty and coworkers, 2024; Popa and coworkers, 2024). Not all potential markers of hemolysis (ie, haptoglobin) were analyzed, because this was not the focus of our study. Although the increase of LDH in T1 may indicate hemolysis as a side effect of ablation, the increase of LDH levels in T2 could be a marker of atrial myocardial loss because of ablation.

PFA using a pentaspline catheter appears safe showing no significant risk of AKI despite the significant increase of hemolysis markers irrespective of the number of applications. In addition, no dose-dependent increase in hemolysis markers was observed across the different PFA modalities in our study setup. Further research involving a larger patient cohort and varying PFA application forms and doses is needed to confirm these findings and to assess their implications for the safety and efficacy of AF treatment.

Conclusion

The main findings of our study are as follows:

1. A significant increase in bilirubin and LDH levels after ablation for all groups.

2. A significant increase in potassium levels after ablation for PFA (PFA-8, PFA-16, and PFA-foc).
3. No significant increase in serum creatinine in any group.

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