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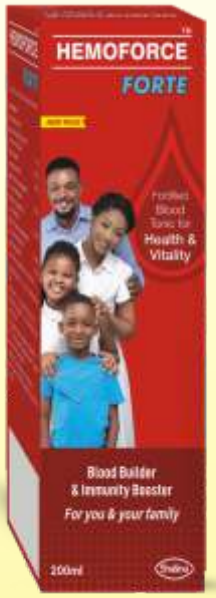

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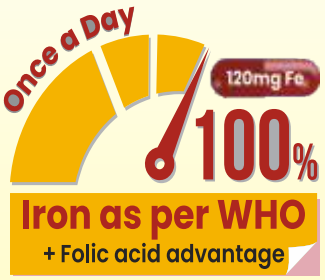

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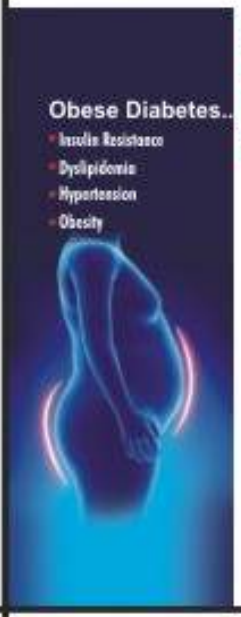

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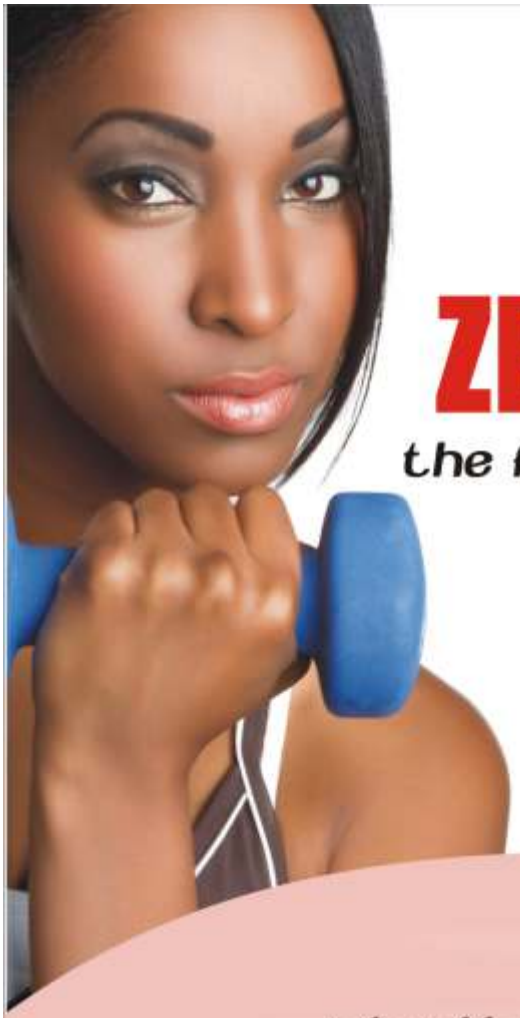

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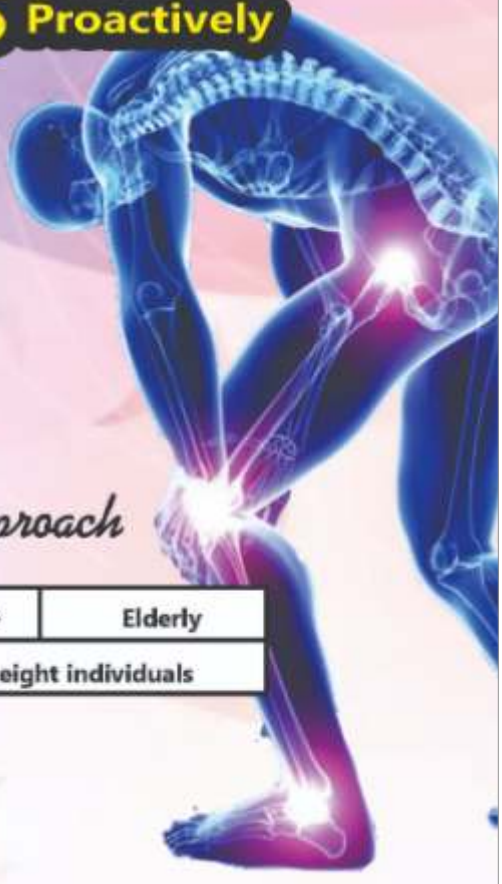

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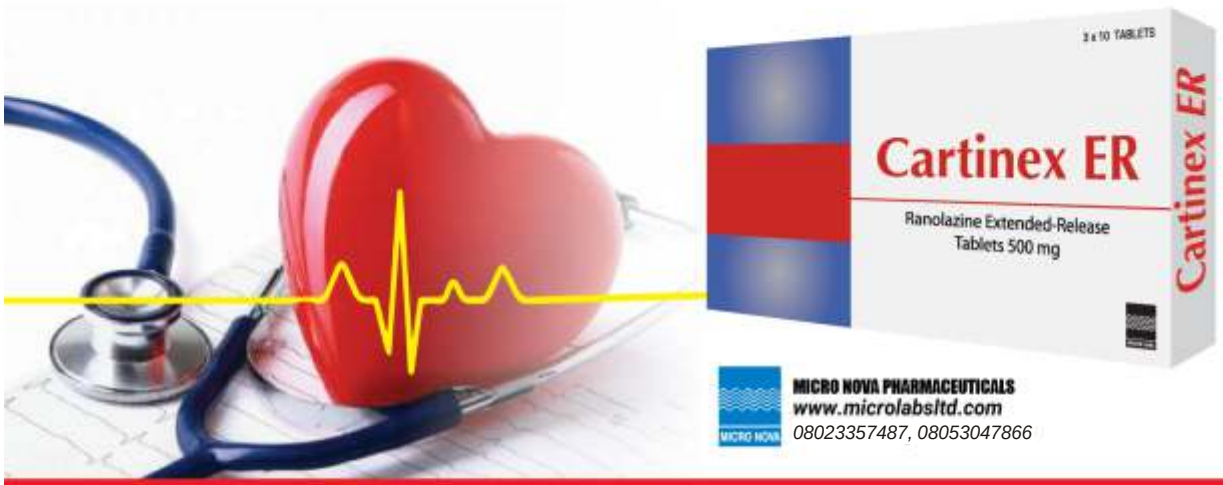

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Duration of cardiopulmonary resuscitation and outcomes for adults with in-hospital cardiac arrest: retrospective cohort study

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Abstract

Objective
To quantify time dependent probabilities of outcomes in patients after in-hospital cardiac arrest as a function of duration of cardiopulmonary resuscitation, defined as the interval between start of chest compression and the first return of spontaneous circulation or termination of resuscitation.

Design
Retrospective cohort study.

Setting
Multicenter prospective in-hospital cardiac arrest registry in the United States.

Participants
348 996 adult patients (≥18 years) with an index in-hospital cardiac arrest who received cardiopulmonary resuscitation from 2000 through 2021.

Main outcome measures
Survival to hospital discharge and favorable functional outcome at hospital discharge, defined as a cerebral performance category score of 1 (good cerebral performance) or 2 (moderate cerebral disability). Time dependent probabilities of subsequently surviving to hospital discharge or having favorable functional outcome if patients pending the first return of spontaneous circulation at each minute received further cardiopulmonary resuscitation beyond the time point were estimated, assuming that all decisions on termination of resuscitation were accurate (that is, all patients with termination of resuscitation would have invariably failed to survive if cardiopulmonary resuscitation had continued for a longer period of time).

Results
Among 348 996 included patients, 233 551 (66.9%) achieved return of spontaneous circulation with a median interval of 7 (interquartile range 3-13) minutes between start of chest compressions and first return of spontaneous circulation, whereas 115 445 (33.1%) patients did not achieve return of spontaneous circulation with a median interval of 20 (14-30) minutes between start of chest compressions and termination of resuscitation. 78 799 (22.6%) patients survived to hospital discharge. The time dependent probabilities of survival and favorable

functional outcome among patients pending return of spontaneous circulation at one minute's duration of cardiopulmonary resuscitation were 22.0% (75 645/343 866) and 15.1% (49 769/328 771), respectively. The probabilities decreased over time and were <1% for survival at 39 minutes and <1% for favorable functional outcome at 32 minutes' duration of cardiopulmonary resuscitation.

Conclusions

This analysis of a large multicenter registry of in-hospital cardiac arrest quantified the time dependent probabilities of patients' outcomes in each minute of duration of cardiopulmonary resuscitation. The findings provide resuscitation teams, patients, and their surrogates with insights into the likelihood of favorable outcomes if patients pending the first return of spontaneous circulation continue to receive further cardiopulmonary resuscitation.

Introduction

In-hospital cardiac arrest is an important public health problem, affecting approximately 300 000 adults annually in the United States, with a high mortality rate.[12] The survival rate after in-hospital cardiac arrest in the US improved from 2000 to 2010 and has remained plateaued after 2010, with approximately 25% of patients surviving to hospital discharge.[3,4]

Achieving return of spontaneous circulation is the first step toward long term survival and favorable functional recovery. However, for nearly half of patients with in-hospital cardiac arrest, resuscitative efforts are terminated without achievement of return of spontaneous circulation.[5] When patients do not achieve return of spontaneous circulation despite cardiopulmonary resuscitation, clinical providers face challenges in deciding how long to continue cardiopulmonary resuscitation. For patients with out-of-hospital cardiac arrest, previous studies showed that longer duration of pre-hospital cardiopulmonary resuscitation before return of spontaneous circulation was associated with poor outcomes for patients.[6,7,8,9,10] However, the association of duration of cardiopulmonary resuscitation with patients' outcomes has not been fully investigated for in-hospital cardiac arrest. The 2020 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations of the Education, Implementation, and Teams Task Force was unable to make recommendations on when to terminate cardiopulmonary resuscitation for in-hospital cardiac arrest.[11] This highlights existing gaps in knowledge and the importance of further evaluation of the effect of duration of cardiopulmonary resuscitation on patients' outcomes after in-hospital cardiac arrest.

Our primary objective was to quantify the time dependent probabilities of favorable outcomes as a function of duration of cardiopulmonary resuscitation among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation who later attained return of spontaneous circulation or had termination of resuscitation. Our secondary objective was to quantify the time dependent probabilities of favorable outcomes as a function of duration of cardiopulmonary resuscitation among patients who had the first return of spontaneous circulation before or at each time point. We also did stratified analyses to investigate whether clinical features and patients' phenotypes modified the association between duration of cardiopulmonary resuscitation and favorable outcomes.

Methods

Study design and setting

This is an analysis of the Get With The Guidelines—Resuscitation (GWTG-R) registry, a multicenter prospective quality improvement registry of in-hospital cardiac arrest in the US. The GWTG programs are provided by the American Heart Association. Details of the registry were previously reported elsewhere.[12] Data are collected on all patients with in-hospital cardiac arrest in the participating hospitals who did not have existing do not resuscitate orders and who received cardiopulmonary resuscitation.[4,13] Cardiac arrest was defined as pulselessness requiring chest compression, defibrillation, or both.[4,13] Several case finding methods were used to consecutively collect cases of in-hospital cardiac arrest, including centralized collection of cardiac arrest flow sheets, review of hospital paging systems, and regular checks of cardiopulmonary resuscitation code carts, pharmacy tracer medication records, and hospital billing charges for use of resuscitation medications.[4] Research or quality assurance staff collect information on in-hospital cardiac arrest events from hospital medical records and cardiac arrest documentation forms.[14] The registry used the standardized Utstein template for the definitions of clinical variables and outcomes.[15,16] Data integrity is ensured by rigorous training and certification of hospital staff, use of standardized software with internal data checks, and a periodic re-abstraction process.[4,12,13]

Study participants

We included adult patients (≥18

years) with an index in-hospital cardiac arrest who received cardiopulmonary resuscitation between 2000 and 2021. We excluded patients with extracorporeal membrane oxygenation in place before the start of cardiopulmonary resuscitation, missing data to classify duration of cardiopulmonary resuscitation, do not resuscitate orders before chest compression, missing data on survival to hospital discharge, duration of cardiopulmonary resuscitation greater than 120 minutes,17 termination of resuscitation because of do not resuscitate orders, or extracorporeal membrane oxygenation after the start of cardiopulmonary resuscitation. In an analysis of favorable functional outcome, we further excluded patients with missing functional outcome at hospital discharge.

Exposure

The main exposure was duration of cardiopulmonary resuscitation in minutes, defined as an interval in whole minutes between the start of chest compression and the first return of spontaneous circulation or termination of resuscitation. We defined return of spontaneous circulation as return of adequate pulse by palpation, Doppler, or arterial blood pressure waveform. Data on the start of cardiopulmonary resuscitation, the first return of spontaneous circulation, and termination of resuscitation were initially recoded at the time of the in-hospital cardiac arrest events by the clinical team and subsequently entered onto the GWTG-R database by research or quality assurance staff.[14]

Outcome measures

Our outcome measures were survival to hospital discharge and favorable functional outcome at hospital discharge, defined as cerebral performance category (CPC) score 1 or 2.[16] The CPC is a 5 point functional scale; a CPC score of 1 represents good cerebral performance, 2 represents moderate cerebral disability, 3 represents severe cerebral disability, 4 represents coma or vegetative state, and 5 represents brain death.[16,18,19]

Statistical analysis

We stratified patients by presence or absence of return of spontaneous circulation and reported characteristics of patients and cardiac arrests. We also reported differences in these characteristics with standardized mean differences between patients with and without missing duration of cardiopulmonary resuscitation, survival to hospital discharge, or functional outcome at hospital discharge. We considered an absolute standardized mean difference within 0.25 to be a small difference.[20]

Cumulative proportion of patients achieving first return of spontaneous circulation over time stratified by patients with outcomes

Using the Kaplan-Meier estimate, we constructed simple curves of the cumulative proportion of patients achieving the first return of spontaneous circulation over time, stratified by survival (among patients who survived to hospital discharge or among patients who had return of spontaneous circulation and subsequently died before hospital discharge) and functional outcome (among patients with favorable functional outcome (CPC score 1 or 2) at hospital discharge, among patients with unfavorable functional outcome (CPC score 3 or 4) at hospital discharge, or among patients who had return of spontaneous circulation and subsequently died before hospital discharge). Using the Greenwood formula for the estimated standard error of the Kaplan-Meier estimate, we also estimated the 95th and 99th centiles of duration of cardiopulmonary resuscitation for each stratified curve with 95% confidence intervals.

Time dependent probabilities of outcomes among patients pending first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation

We calculated time dependent probabilities for the outcomes as a function of duration of cardiopulmonary resuscitation. Firstly, we calculated time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation. The numerator was the number of patients who were pending the first return of spontaneous circulation at each minute and subsequently had each outcome. The denominator was the number of patients pending the first return of spontaneous circulation at each minute. This time dependent probability represented the probability of subsequently surviving to hospital discharge or having favorable functional outcome if the patients pending the first return of spontaneous circulation at that time point received further cardiopulmonary resuscitation beyond the time point (supplementary methods and figure A).

We calculated two time dependent probabilities of each outcome among patients pending the first return of spontaneous circulation at each minute, as we defined two denominators including and excluding patients with termination of resuscitation before

or at each time point (supplementary methods and figure A). As a primary analysis, we included patients who had termination of resuscitation before or at each minute's duration of cardiopulmonary resuscitation in the denominator (supplementary methods and figure A). Use of this denominator provides probabilities of having outcomes among the overall study population if patients pending the first return of spontaneous circulation had further cardiopulmonary resuscitation beyond that time point, assuming that all decisions on termination of resuscitation were accurate and that the patients who had termination of resuscitation never had outcomes, even if the patients would have had longer duration of cardiopulmonary resuscitation beyond the time point of termination of resuscitation. We reported duration of cardiopulmonary resuscitation when this probability became less than 1%, using traditional medical futility, a likelihood of survival of less than 1%.[21,22]

As a sensitivity analysis, another denominator included only patients who were undergoing cardiopulmonary resuscitation at each minute pending the first return of spontaneous circulation, and excluding patients who had termination of resuscitation before or at each minute (supplementary methods and figure A). This denominator treated termination of resuscitation as a censoring event that is not informative on subsequent time dependent probabilities. Therefore, as duration of cardiopulmonary resuscitation increased, this denominator included only patients who were undergoing cardiopulmonary resuscitation and represented a selected population for whom the resuscitation team chose to provide prolonged cardiopulmonary resuscitation.

Time dependent probabilities of outcomes among patients who had first return of spontaneous circulation before or at each minute's duration of cardiopulmonary resuscitation

Secondly, we calculated the time dependent probability for each outcome among patients who had the first return of spontaneous circulation before or at each time point (supplementary methods and figure A). This time dependent probability quantified the probability of surviving to hospital discharge or having favorable functional outcome once patients achieved the first return of spontaneous circulation before or at each time point. We carried out pointwise estimation of 95% confidence interval of each time dependent probability on the basis of the variance of binomial distribution.

Stratified analyses of time dependent probabilities

Additionally, we defined clinical features as age group, witness status, and initial rhythm. To evaluate whether the time dependent probabilities differed across clinical features of cardiac arrest, we stratified the time dependent probability curves on the basis of age group (<60 years, 60-79 years, or $\geq$ 80 years), witness status (witnessed or unwitnessed), and initial rhythm (shockable or non-shockable rhythm).[23]

We defined patients' phenotype as each combination of age group, witness status, and initial rhythm. To investigate whether phenotypes of patients affect the relation between duration of cardiopulmonary resuscitation and outcomes, we plotted the time dependent probabilities for each phenotype.

Subgroup analysis

As the dataset included in-hospital cardiac arrests from 2000 through 2021, we did subgroup analyses including only patients from 2011 through 2021 to evaluate the recent in-hospital cardiac arrest data. We used Stata 16.1 and R software, version 4.0.2, for all statistical analyses

Patient and public involvement

No patients or members of the public were involved in setting the research question or the outcome measures, nor were they involved in developing plans for the design or implementation of the study or asked to advise on interpretation or writing up of results.

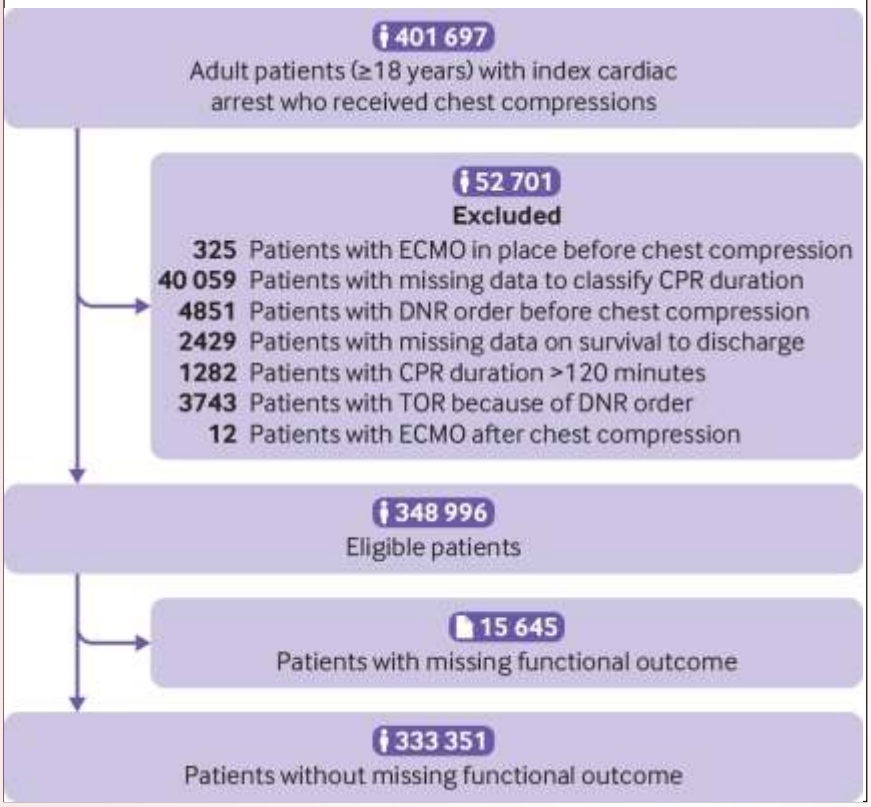
Results

We identified 401 697 patients with an index in-hospital cardiac arrest who received cardiopulmonary resuscitation (fig 1). After excluding those who met the exclusion criteria, we included 348 996 patients in our study. We further excluded 15 645 patients who were missing functional outcome at hospital discharge from the analysis of functional outcome.

Table 1 and table 2 show characteristics of patients and cardiac arrests. Among 348 996 included patients, 233 551 (66.9%) achieved return of spontaneous circulation and 78 799 (22.6%) survived to hospital discharge. The median interval between the start of chest compressions and the first return of spontaneous circulation was 7 (interquartile range 3-13) minutes among patients who had return of spontaneous circulation. The median interval between start of chest compressions and termination of resuscitation was 20 (14-30)

Fig 1

Patient flow. CPR=cardiopulmonary resuscitation; DNR=do not resuscitate; ECMO=extracorporeal membrane oxygenation; TOR=termination of resuscitation



minutes among patients who did not have return of spontaneous circulation. Among 333 351 patients without missing functional outcome at hospital discharge, 52 104 (15.6%) had favorable functional outcome. See supplementary table A for characteristics of patients and cardiac arrests in patients with and without missing duration of cardiopulmonary resuscitation, survival to hospital discharge, or functional outcome at hospital discharge. For all of characteristics of patients and most characteristics of cardiac arrests, the standardized differences were within 0.25, and the characteristics were similar.

Cumulative proportion of patients achieving first return of spontaneous circulation over time stratified by outcomes

Figure 2 shows the cumulative proportion of patients achieving the first return of spontaneous circulation, stratified by patients' outcomes. Almost all (99%) patients who survived to hospital discharge had the first return of spontaneous circulation within 44 (95% confidence interval 43 to 45) minutes' duration of CPR (fig 2, top). Almost all (99%) patients who had favorable functional outcome at hospital discharge had the first return of spontaneous circulation within 43 (41 to 44) minutes (fig 2, bottom).

Time dependent probabilities of outcomes among patients pending first return of spontaneous circulation at each minute's duration of CPR

We present time dependent probabilities of survival to hospital discharge (fig 3, fig 4, and fig 5) and favorable functional outcome at hospital discharge (fig 6, fig 7, and fig 8) among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation for overall patients and each clinical feature and patient phenotype, using the denominator including patients who had termination of resuscitation before or at each time point (primary analysis). Among overall patients with in-hospital cardiac arrest, the probabilities of survival and favorable functional outcome among those pending the first return of spontaneous circulation at 1 minute's duration of cardiopulmonary resuscitation were 22.0% and 15.1%, respectively (fig 3, top; fig 6, top). As duration of cardiopulmonary resuscitation increased, the probabilities of survival and favorable functional outcome decreased and plateaued at 0.3-0.9% and 0.1-0.5% between 40 minutes and 60 minutes, respectively. The probabilities of survival at 39 minutes' and favorable functional status at 32 minutes' duration of cardiopulmonary resuscitation were less than 1% (supplementary table B). In terms of clinical features, age younger than 60 years, witnessed arrest, and initial shockable rhythm showed the higher estimates of the time dependent probabilities of survival (fig 3, bottom; fig 4) and favorable functional outcome (fig 6, bottom; fig 7). Across clinical features, stratification by initial rhythm showed the largest change in the time dependent probabilities for each outcome (fig 4, bottomt; fig 7, bottom), and shockable rhythm had the longest duration of cardiopulmonary resuscitation before the time dependent probabilities of survival and functional outcome became less than 1% (supplementary

Table 1 Patients' characteristics. Values are numbers (percentages) unless stated otherwise

Characteristics	All patients (n=348 996) (n=115 445)	Patients without ROSC (n=233 551)	Patients with ROSC
Median (IQR) age, years	67 (56-77)	68 (56-79)	67 (56-76)
Sex:			
Male	203 883 (58.4)	69 495 (60.2)	134 388 (57.5)
Female	145 083 (41.6)	45 935 (39.8)	99 148 (42.5)
Unknown	24 (0.0)	10 (0.0)	14 (0.0)
Race:			
White	239 185 (68.5)	78 403 (67.9)	160 782 (68.8)
Black	76 329 (21.9)	25 518 (22.1)	50 811 (21.8)
Other*	11 316 (3.2)	3711 (3.2)	7605 (3.3)
Unknown	22 166 (6.4)	7813 (6.8)	14 353 (6.1)
Illness category:			
Medical			
Cardiac	123 297 (35.3)	40 251 (34.9)	83 046 (35.6)
Non-cardiac	153 957 (44.1)	52 934 (45.9)	101 023 (43.3)
Surgical			
Cardiac	21 351 (6.1)	5637 (4.9)	15 714 (6.7)
Non-cardiac	36 747 (10.5)	11 785 (10.2)	24 962 (10.7)
Trauma	12 288 (3.5)	4440 (3.8)	7848 (3.4)
Other†	1078 (0.3)	309 (0.3)	769 (0.3)
Unknown	278 (0.08)	89 (0.08)	189 (0.08)
Pre-existing condition:			
Cardiac			
History of myocardial infarction	48 428 (13.9)	15 635 (13.5)	32 793 (14.0)
Myocardial infarction, this admission	49 148 (14.1)	15 383 (13.3)	33 765 (14.5)
History of heart failure	71 367 (20.4)	22 369 (19.4)	48 998 (21.0)
Heart failure, this admission	51 101 (14.6)	15 914 (13.8)	35 187 (15.1)
Non-cardiac			
Respiratory insufficiency	147 083 (42.1)	46 149 (40.0)	100 934 (43.2)
Diabetes mellitus	107 010 (30.7)	31 775 (27.5)	75 235 (32.2)
Renal insufficiency	114 027 (32.7)	35 361 (30.6)	78 666 (33.7)
Metastatic or hematologic malignancy	37 304 (10.7)	13 625 (11.8)	23 679 (10.1)
Hypotension or hypoperfusion	89 250 (25.6)	29 544 (25.6)	59 706 (25.6)
Pneumonia	46 234 (13.2)	14 501 (12.6)	31 733 (13.6)
Baseline depression in CNS function	32 032 (9.2)	11 172 (9.7)	20 860 (8.9)
Metabolic or electrolyte abnormality	68 270 (19.6)	20 557 (17.8)	47 713 (20.4)
Sepsis	45 677 (13.1)	15 016 (13.0)	30 661 (13.1)
Acute CNS non-stroke event	28 326 (8.1)	8417 (7.3)	19 909 (8.5)
Hepatic insufficiency	26 617 (7.6)	8417 (7.3)	18 200 (7.8)
Acute stroke	12 675 (3.6)	4082 (3.5)	8593 (3.7)
Major trauma	15 107 (4.3)	5255 (4.6)	9852 (4.2)

CNS=central nervous system; IQR=interquartile range; ROSC=return of spontaneous circulation.

* Asian, Native Americans/Alaska Natives, "others," and Native Hawaiians/Pacific Islanders.

† Obstetrics and "other."

Table 2 Characteristics of cardiac arrests. Values are numbers (percentages) unless stated otherwise

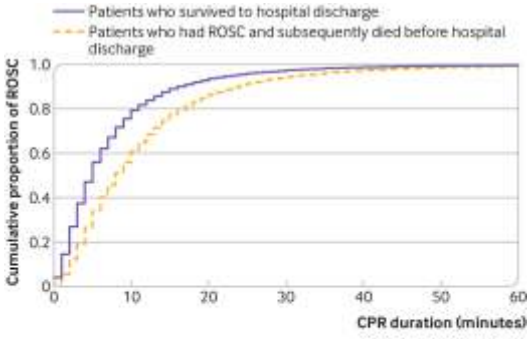
Characteristics	All patients (n=348 996) (n=115 445)	Patients without ROSC (n=233 551)	Patients with ROSC
Year of cardiac arrest:			
2000-05	57 554 (16.5)	26 979 (23.4)	30 575 (13.1)
2006-10	81 615 (23.4)	30 228 (26.2)	51 387 (22.0)
2011-15	92 523 (26.5)	27 415 (23.7)	65 108 (27.9)
2016-21	117 304 (33.6)	30 823 (26.7)	86 481 (37.0)
Interventions in place at time of cardiac arrest:			
Non-invasive assisted ventilation	1610 (0.5)	413 (0.4)	1197 (0.5)
Mechanical ventilation	74 845 (21.4)	21 742 (18.8)	53 103 (22.7)
Dialysis	11 241 (3.2)	3716 (3.2)	7525 (3.2)
Implantable cardiac defibrillator	5978 (1.7)	1968 (1.7)	4010 (1.7)
Intra-arterial catheter	35 770 (10.2)	11 061 (9.6)	24 709 (10.6)
Electrocardiogram monitor	283 494 (81.2)	89 291 (77.3)	194 203 (83.2)
Pulse oximeter	245 878 (70.5)	76 983 (66.7)	168 895 (72.3)
Vasoactive agents	86 646 (24.8)	31 213 (27.0)	55 433 (23.7)
Antiarrhythmic agents	8571 (2.5)	2672 (2.3)	5899 (2.5)
Location of cardiac arrest:			
Emergency department	42 507 (12.2)	14 092 (12.2)	28 415 (12.2)
Floor with telemetry or step-down unit	54 914 (15.7)	17 472 (15.1)	37 442 (16.0)
Floor without telemetry	55 643 (15.9)	21 609 (18.7)	34 034 (14.6)
Intensive care unit or coronary care unit	163 799 (46.9)	53 014 (45.9)	110 785 (47.4)
Operating room, post-anesthesia care unit, cardiac catheterization laboratory, or diagnostic/interventional unit	24 781 (7.1)	6733 (5.8)	18 048 (7.7)
Other*	7198 (2.1)	2469 (2.1)	4729 (2.0)
Unknown	154 (0.04)	56 (0.05)	98 (0.04)
Witness status:			
Witnessed	297 826 (85.3)	93 613 (81.1)	204 213 (87.4)
Unwitnessed	51 170 (14.7)	21 832 (18.9)	29 338 (12.6)
First documented rhythm,:			
Asystole	99 933 (28.6)	40 971 (35.5)	58 962 (25.2)
Pulseless electrical activity	167 779 (48.1)	54 178 (46.9)	113 601 (48.6)
Ventricular fibrillation	33 100 (9.5)	9097 (7.9)	24 003 (10.3)
Pulseless ventricular tachycardia	23 337 (6.7)	5164 (4.5)	18 173 (7.8)
Unknown	24 847 (7.1)	6035 (5.2)	18 812 (8.1)
Epinephrine administration	310 166 (88.9)	111 804 (96.8)	198 362 (84.9)
Median (IQR) interval between start of chest compression and first epinephrine administration, minutes	1 (0-3)	2 (0-4)	1 (0-3)
Endotracheal intubation	174 504 (50.0)	61 100 (52.9)	113 404 (48.6)
Median (IQR) interval between start of chest compression and endotracheal intubation, , minutes	5 (2-9)	5 (2-9)	5 (2-9)

IQR=interquartile range; ROSC=return of spontaneous circulation.

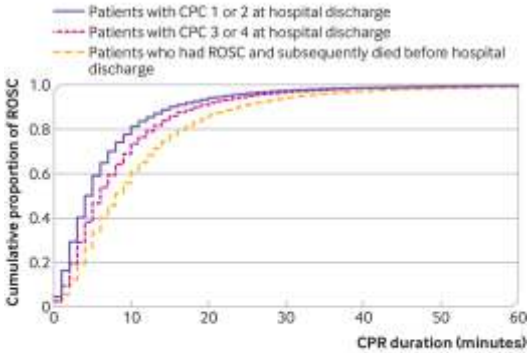
* Ambulatory and outpatient areas; delivery suite; rehabilitation, skilled nursing, and mental health facilities; same day surgical areas; and “other.”

Fig 2

Cumulative proportion of patients achieving first return of spontaneous circulation (ROSC), stratified by patients with outcomes: survival to hospital discharge (top) and functional outcome at hospital discharge (bottom). CI=confidence interval; CPC=cerebral performance category; CPR=cardiopulmonary resuscitation



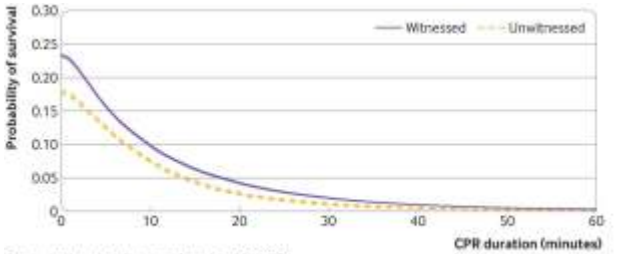
	95th centile	99th centile
Patients who survived to hospital discharge	23 (23 to 24)	44 (43 to 45)
Patients who had ROSC and subsequently died before hospital discharge	32 (32 to 32)	55 (55 to 56)



	95th centile	99th centile
Patients with CPC 1 or 2 at hospital discharge	22 (22 to 23)	43 (41 to 44)
Patients with CPC 3 or 4 at hospital discharge	25 (25 to 26)	47 (44 to 50)
Patients who had ROSC and subsequently died before hospital discharge	32 (32 to 32)	56 (55 to 57)

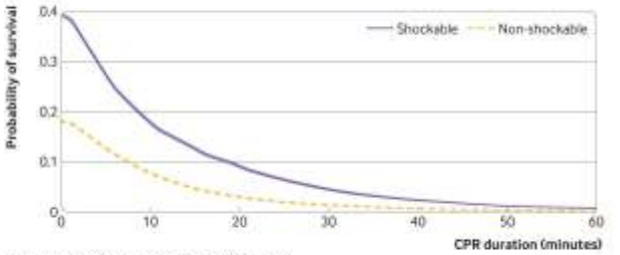
Fig. 3

Time dependent probability of survival to hospital discharge with 95% confidence intervals among patients pending first return of spontaneous circulation at each time point for all patients (top) and stratified by age group (bottom). Denominators included patients who were undergoing cardiopulmonary resuscitation (CPR) pending first return of spontaneous circulation and patients who had termination of resuscitation before or at each time point



Survival to hospital discharge/receiving CPR (%)

	0	10	20	30	40	50	60
Witnessed	66 812/ 293 132 (22.8)	16 550/ 166 791 (9.9)	5056/ 118 432 (4.3)	2054/ 104 024 (2.0)	961/ 98 512 (1.0)	494/ 96 135 (0.5)	290/ 94 989 (0.3)
Unwitnessed	8833/ 50 734 (17.4)	2724/ 36 104 (7.5)	713/ 26 429 (2.7)	253/ 23 419 (1.1)	105/ 22 530 (0.5)	57/ 22 156 (0.3)	30/ 21 991 (0.1)



Survival to hospital discharge/receiving CPR (%)

	0	10	20	30	40	50	60
Shockable	21 091/ 54 954 (38.4)	5031/ 28 068 (17.9)	1790/ 19 529 (9.2)	742/ 16 607 (4.5)	355/ 15 352 (2.3)	179/ 14 816 (1.2)	104/ 14 561 (0.7)
Non-shockable	46 948/ 264 835 (17.7)	12 753/ 163 252 (7.8)	3542/ 117 388 (3.0)	1381/ 103 985 (1.3)	636/ 99 259 (0.6)	335/ 97 230 (0.3)	197/ 96 272 (0.2)

table B). Figure 5 shows time dependent probabilities of survival and figure 8 shows time dependent probabilities of favorable functional outcome for patient phenotypes. Across patient phenotypes, patients who were younger than 60 years, had witnessed arrest, and had initial shockable rhythm showed the highest point estimates of the time dependent probabilities of survival (fig 5) and favorable functional outcome (fig 8) and the longest duration of cardiopulmonary resuscitation before the time dependent probabilities became less than 1% (supplementary table B).

Supplementary figures B and C show the time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation, using the denominator excluding patients who had termination of resuscitation before or at each time point (sensitivity analysis). Among overall patients with in-hospital cardiac arrest, the time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at 1 minute's duration of cardiopulmonary resuscitation were 22.0% and 15.2%, respectively. As duration of cardiopulmonary resuscitation increased from 1 to 20 minutes, the probabilities decreased but then plateaued at 5.4-6.3% for survival and 3.2-3.8% for favorable functional outcome between 20 minutes and 60 minutes.

Time dependent probabilities of

Fig 4

Time dependent probability of survival to hospital discharge with 95% confidence intervals among patients pending first return of spontaneous circulation at each time point, stratified by witness status (top) and initial rhythm (bottom). Denominators included patients who were undergoing cardiopulmonary resuscitation (CPR) pending first return of spontaneous circulation and patients who had termination of resuscitation before or at each time point

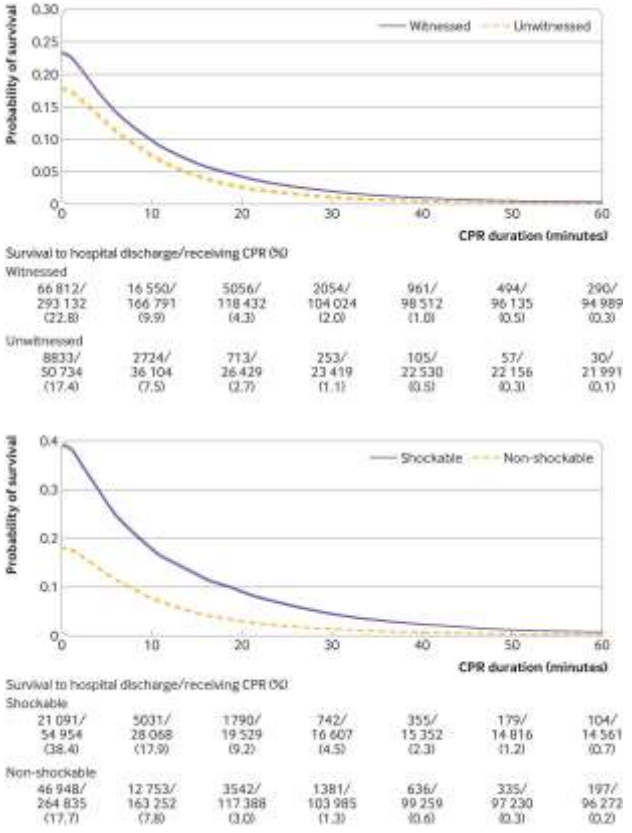
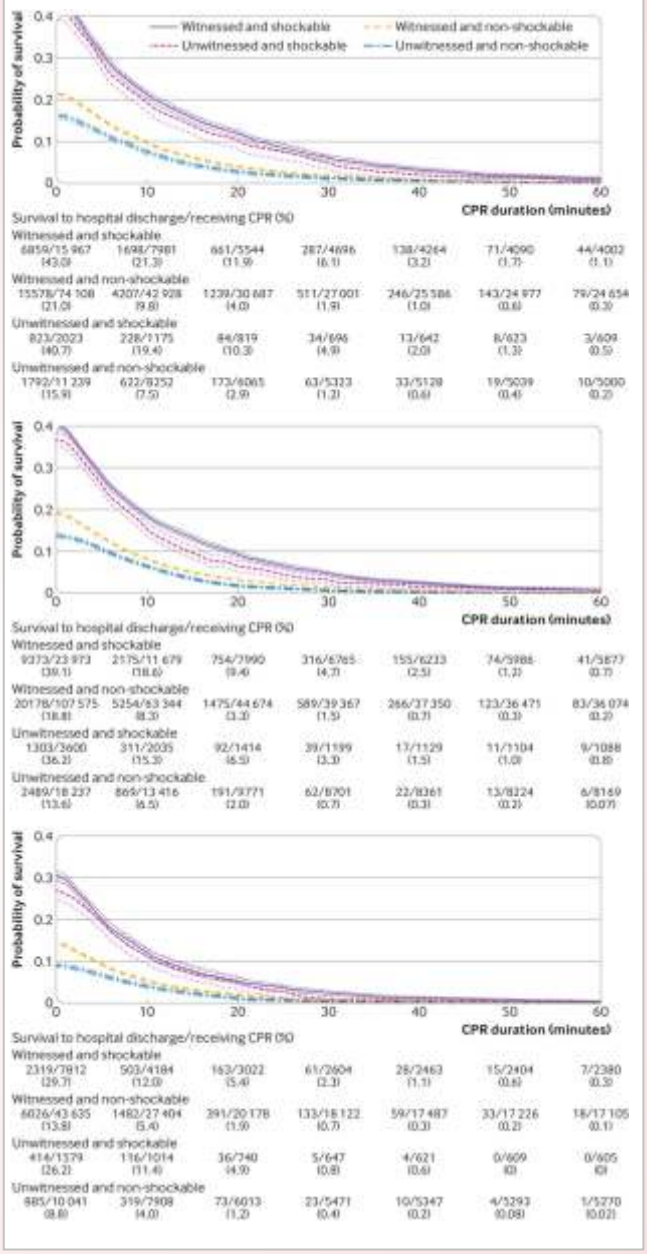


Fig 5

Time dependent probability of survival to hospital discharge with 95% confidence intervals among patients pending first return of spontaneous circulation at each time point. Combination of witness status and initial rhythm among age <60 years (top), age 60-79 years (middle), and age ≥80 years (bottom). Denominators included patients who were undergoing cardiopulmonary resuscitation (CPR) pending first return of spontaneous circulation and patients who had termination of resuscitation before or at each time point



outcomes among patients who had first return of spontaneous circulation before or at each minute's duration of cardiopulmonary resuscitation

In the supplement, we present time dependent probabilities of survival (figure D) and favorable functional outcome (figure E) among patients who had the first return of spontaneous circulation before or at each time point for overall patients and for each clinical feature and patient phenotype. Among clinical features, younger age group and initial shockable rhythm consistently showed higher time dependent probability of survival (supplementary figures D2 and D4) and favorable functional outcome (supplementary figures E2 and E4), and witness status showed crossover of the probability for each outcome (supplementary figures D3 and E3).

Subgroup analysis

The subgroup analysis including the subset of patients with in-hospital cardiac arrest between 2011 and 2021 is shown in supplementary figures F-L and table B. Across most of the clinical

Fig 6

Time dependent probability of favorable functional outcome at hospital discharge with 95% confidence intervals among patients pending first return of spontaneous circulation at each time point for all patients (top) and stratified by age group (bottom). Denominators included patients who were undergoing cardiopulmonary resuscitation (CPR) pending first return of spontaneous circulation and patients who had termination of resuscitation before or at each time point

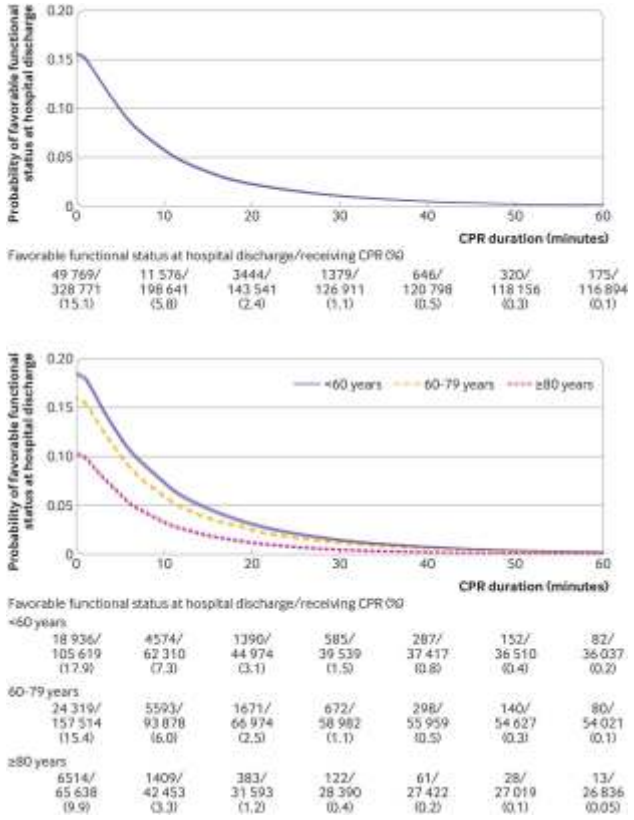
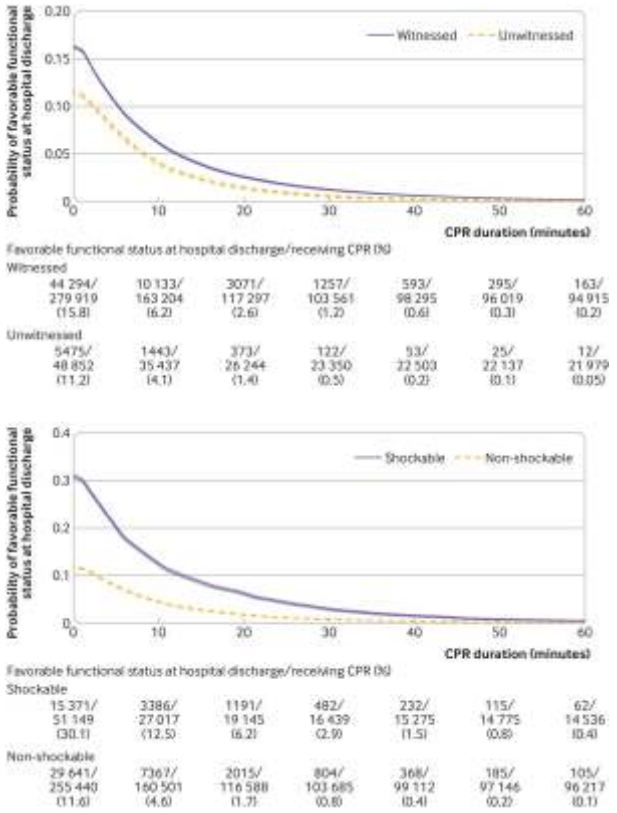


Fig 7

Time dependent probability of favorable functional outcome at hospital discharge with 95% confidence intervals among patients pending first return of spontaneous circulation at each time point, stratified by witness status (top) and initial rhythm (bottom). Denominators included patients who were undergoing cardiopulmonary resuscitation (CPR) pending first return of spontaneous circulation and patients who had termination of resuscitation before or at each time point



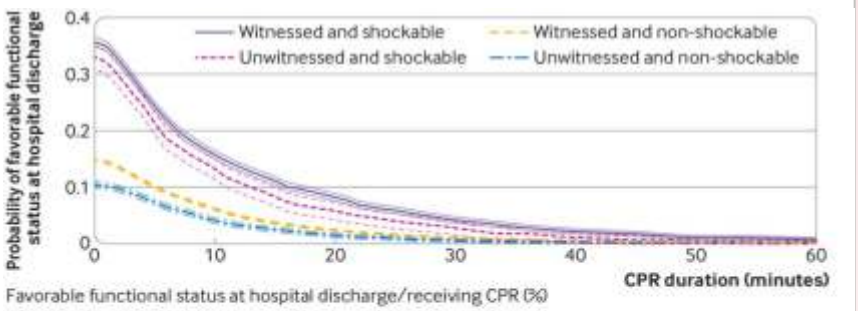
features and patient phenotypes, the durations of cardiopulmonary resuscitation before the time dependent probabilities of survival and functional outcome among patients pending the first return of spontaneous circulation before or at each time point became less than 1% were longer in the subgroup of patients between 2011 and 2021 than in the overall study population between 2000 and 2021 (supplementary table B).

Discussion

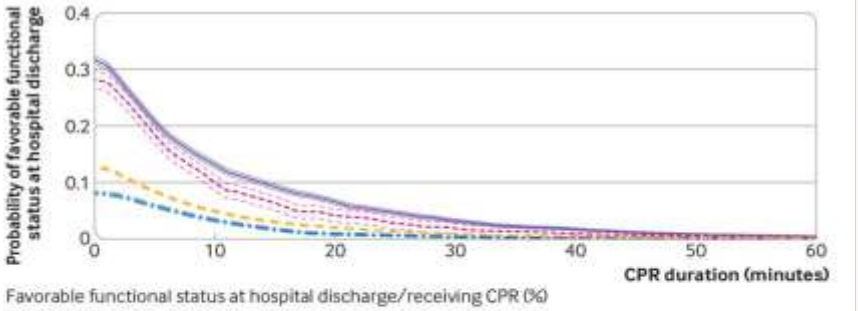
In this analysis of a large multicenter prospective registry of in-hospital cardiac arrest in the United States between 2000 and 2021, we quantified the time dependent probabilities of survival to hospital discharge and favorable functional outcome at hospital discharge as a function of duration of cardiopulmonary resuscitation. We found that 99% of patients who eventually survived to hospital discharge and who had favorable functional outcome at hospital discharge achieved the first return of spontaneous circulation within 44 minutes' and 43 minutes' duration of cardiopulmonary resuscitation, respectively. The time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation using the denominator that included patients who had termination of resuscitation before or at each time point decreased and plateaued at less than 1% as duration of cardiopulmonary resuscitation increased beyond 39 minutes and 32 minutes, respectively. By contrast, the time dependent probabilities using the denominator that excluded patients who had termination of resuscitation before or at each time point plateaued above 5% for survival and 3% for favorable functional outcome as duration of cardiopulmonary resuscitation increased.

Fig 8

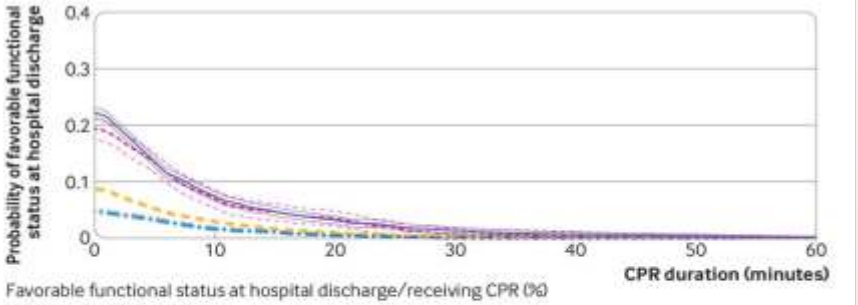
Time dependent probability of favorable functional outcome at hospital discharge with 95% confidence intervals among patients pending first return of spontaneous circulation at each time point. Combination of witness status and initial rhythm among age <60 years (top), age 60-79 years (middle), and age ≥80 years (bottom). Denominators included patients who were undergoing cardiopulmonary resuscitation (CPR) pending first return of spontaneous circulation and patients who had termination of resuscitation before or at each time point



Favorable functional status at hospital discharge/receiving CPR (%)						
Witnessed and shockable						
5139/14 764 (34.8)	1195/7654 (15.6)	446/5405 (8.3)	195/4634 (4.2)	93/4233 (2.2)	48/4074 (1.2)	30/3994 (0.8)
Witnessed and non-shockable						
10 248/71 074 (14.4)	2577/42 060 (6.1)	716/30 405 (2.4)	303/26 886 (1.1)	149/25 529 (0.6)	81/24 940 (0.3)	43/24 631 (0.2)
Unwitnessed and shockable						
599/1875 (31.9)	149/1126 (13.2)	46/795 (5.8)	19/686 (2.8)	7/638 (1.1)	3/620 (0.5)	1/608 (0.2)
Unwitnessed and non-shockable						
1119/10 869 (10.3)	331/8107 (4.1)	92/6021 (1.5)	27/5306 (0.5)	16/5120 (0.3)	8/5033 (0.2)	5/4996 (0.1)



Favorable functional status at hospital discharge/receiving CPR (%)						
Witnessed and shockable						
6840/22 272 (30.7)	1476/11 215 (13.2)	517/7830 (6.6)	210/6696 (3.1)	102/6200 (1.6)	50/5972 (0.8)	24/5868 (0.4)
Witnessed and non-shockable						
12 828/103 586 (12.4)	3076/62 225 (4.9)	885/44 363 (2.0)	366/39 251 (0.9)	156/37 291 (0.4)	71/36 443 (0.2)	45/36 052 (0.1)
Unwitnessed and shockable						
931/3361 (27.7)	194/1964 (9.9)	58/1390 (4.2)	22/1187 (1.9)	10/1124 (0.9)	5/1099 (0.5)	3/1083 (0.3)
Unwitnessed and non-shockable						
1421/17 680 (8.0)	439/13 207 (3.3)	88/9725 (0.9)	31/8688 (0.4)	10/8354 (0.1)	7/8219 (0.09)	3/8167 (0.04)



Favorable functional status at hospital discharge/receiving CPR (%)						
Witnessed and shockable						
1579/7373 (21.4)	302/4068 (7.4)	98/2990 (3.3)	32/2589 (1.2)	17/2459 (0.7)	9/2401 (0.4)	4/2378 (0.2)
Witnessed and non-shockable						
3574/42 375 (8.4)	810/27 070 (3.0)	202/20 079 (1.0)	70/18 089 (0.4)	33/17 472 (0.2)	17/17 218 (0.1)	9/17 101 (0.05)
Unwitnessed and shockable						
283/1504 (18.8)	70/990 (7.1)	26/735 (3.5)	4/647 (0.6)	43/621 (0.5)	0/609 (0)	0/605 (0)
Unwitnessed and non-shockable						
451/9856 (4.6)	134/7832 (1.7)	32/5995 (0.5)	7/5465 (0.1)	4/5346 (0.07)	1/5293 (0.02)	0/5270 (0)

Comparison with other studies

A previous observational study using the GWTG-R registry showed that duration of resuscitative efforts before termination of resuscitation varied across hospitals, and resuscitation at hospitals with longer resuscitative efforts before termination of resuscitation was associated with an increased chance of survival to hospital discharge.[24] Another study using the GWTG-R registry reported that patients with higher predicted survival had longer duration of resuscitative efforts before termination of resuscitation.[25] However, scarce previous work has evaluated the association between duration of cardiopulmonary resuscitation at the patient level and patients' outcomes after in-hospital cardiac arrest. A systematic review and meta-analysis in 2019 evaluating pre-arrest and intra-arrest prognostic factors for in-hospital cardiac arrest included only one published study from 1995 and one unpublished study between 2011 and 2018 from the United Kingdom National Cardiac Arrest Audit (UK NCAA) to evaluate the association between duration of cardiopulmonary resuscitation and survival.[26,27] The systematic review and meta-analysis reported that duration of cardiopulmonary resuscitation of more than 15 minutes was associated with decreased odds of survival (adjusted odds ratio 0.06 (95% confidence interval 0.02 to 0.21); adjusted odds ratio 0.13 (0.12 to 0.14) for the UK NCAA data). A Swedish observational study published in 2018 showed the association between longer quarters of duration of cardiopulmonary resuscitation and decreased chance of 30 day survival (adjusted odds ratio 0.69 (0.37 to 1.29) for quarter 2 (duration 3-5 minutes); 0.35 (0.19 to 0.65) for quarter 3 (duration 6-12 minutes); 0.10 (0.05 to 0.20) for

quarter 4 (duration ≥13 minutes)), compared with quarter 1 (duration <2 minutes).[28] A recent observational study of 8727 patients with in-hospital cardiac arrest in 2022, using a national in-hospital cardiac arrest registry in Denmark, reported the association between duration of cardiopulmonary resuscitation and 30 day survival rate (62.0% for quarter 1 (duration <5 minutes), 32.7% for quarter 2 (duration 5-11 minutes), 14.4% for quarter 3 (duration 12-20 minutes), and 8.1% for quarter 4 (duration ≥21 minutes).[29] These previous studies have several methodological limitations. The duration of cardiopulmonary resuscitation was treated as a categorical variable, and odds ratios were reported assuming a linear relation between duration of cardiopulmonary resuscitation and outcomes. Avoiding categorizing a continuous variable is recommended, as this can result in loss of information and is rarely justifiable, compared with analyzing the continuous variable on its continuous scale.[30,31] In addition, our results indicate that duration of cardiopulmonary resuscitation and probabilities of favorable outcomes do not show a linear relation. Our findings extend our understanding of the relation between duration of cardiopulmonary resuscitation and outcomes by quantifying time dependent probabilities of subsequent outcomes when patients did not have the first return of spontaneous circulation and when patients achieved the first return of spontaneous circulation in each minute's duration of cardiopulmonary resuscitation.

In comparison with out-of-hospital cardiac arrest, a retrospective analysis of a multicenter, cluster randomized clinical trial (ROC-PRIMED; Resuscitation Outcomes Consortium Prehospital Resuscitation Using an Impedance Valve and Early Versus Delayed) in the US and Canada showed that 99% of patients who had favorable functional outcome at hospital discharge had return of spontaneous circulation within 37 minutes' duration of cardiopulmonary resuscitation.[10] This is shorter than 43 minutes in our results, which is likely explained by the difference in time to start of cardiopulmonary resuscitation in out-of-hospital and in-hospital settings.

Implications of findings

Firstly, we quantified the time dependent changes in the probabilities of patients' outcomes as a function of duration of cardiopulmonary resuscitation. The time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation provide resuscitation teams, patients, and their surrogates with insights into the likelihood of favorable outcomes if the patient continues to receive cardiopulmonary resuscitation beyond that time point, which is clinically informative for shared decision making to determine whether further cardiopulmonary resuscitation would be beneficial. For example, a pamphlet describing this time dependent probabilities would be helpful for patients' surrogates to provide objective guidance and make decisions on continuing or discontinuing cardiopulmonary resuscitation. On the other hand, the probabilities of survival and favorable functional outcome among patients who had the first return of spontaneous circulation before or at each time point inform resuscitation teams, patients, and their surrogates of the likelihood of favorable outcomes once the patient has achieved return of spontaneous circulation at or by the time point, which is clinically relevant to estimate subsequent outcomes after return of spontaneous circulation.

Secondly, we observed two distinct features of time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation, depending on the denominators used. Notably, these two probabilities are different in two different populations. When the denominator included patients who had termination of resuscitation before or at each time point, the time dependent probabilities of survival and favorable functional outcome decreased and plateaued below traditional medical futility, the likelihood of survival of less than 1% as patients received longer duration of cardiopulmonary resuscitation.[21,22] By contrast, using the denominator that excluded patients who had termination of resuscitation before or at each time point, the probabilities of survival and favorable functional outcome plateaued above 5% and above 3% respectively after 20 minutes' cardiopulmonary resuscitation. This difference could be probably explained by two factors: self-fulfilling prophecy—the treating team used duration of cardiopulmonary resuscitation for decisions to terminate resuscitative efforts—and confounding by indication—only a subset of patients for whom the treating providers believed that prolonged cardiopulmonary resuscitation could be beneficial had prolonged cardiopulmonary resuscitation. Therefore, only highly selected patients were included in the denominator when patients who had termination of resuscitation were excluded. These findings would imply that the decision to terminate resuscitation should not

be solely dependent on duration of cardiopulmonary resuscitation (for example, time points of <1% of survival in supplementary table B) but should be based on clinical judgment of treating providers.

Thirdly, given the time dependent probabilities of outcomes among patients pending the first return of spontaneous circulation at each minute's duration of cardiopulmonary resuscitation across the clinical features and patient phenotypes, in-hospital cardiac arrest with younger age, witnessed, and with initial shockable rhythm would benefit from longer duration of cardiopulmonary resuscitation than those with older age, unwitnessed, and with initially non-shockable rhythm.

Fourthly, in the subgroup analysis that included patients with in-hospital cardiac arrest between 2011 and 2021, we found that, for most of the clinical features and patient phenotypes, the durations of cardiopulmonary resuscitation before the time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each time point with the denominators that included termination of resuscitation became less than 1% were longer than those of the overall study population between 2000 and 2021 (supplementary table B). This may be due to improved post-resuscitation care in the 2011-21 time period.[4,32] This suggests that outcome rates in the study population affect the time dependent probability. As variations in outcomes across participating hospitals in the GWTG-R are known,[33] the time dependent probability of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each time point may vary across the hospitals. Our results should be interpreted as the average time dependent probability of outcomes in the dataset, and the probabilities may not be the same at each hospital.

Unanswered questions and future research

The stratified time dependent probabilities of survival and favorable functional outcome among patients pending the first return of spontaneous circulation at each time point by clinical features and patient phenotypes provided information about who could benefit from prolonged cardiopulmonary resuscitation, as the probabilities of favorable outcomes differed across features and phenotypes. However, other factors in addition to clinical features and patient phenotypes may also be determinants of the outcomes, and further work is warranted to understand such factors that could justify prolonged cardiopulmonary resuscitation.

In our study, the median interval between start of chest compression and termination of resuscitation was 20 (interquartile range 14-30) minutes among patients without return of spontaneous circulation, whereas we found that the probabilities of survival to hospital discharge and favorable functional outcome become less than 1% at 39 minutes' and 32 minutes' duration of cardiopulmonary resuscitation, respectively. Most termination of resuscitation occurred before the time point of traditional medical futility. Further research is needed to evaluate whether patients' outcomes would improve with prolonged cardiopulmonary resuscitation before termination of resuscitation. Our results might generate a clinical equipoise that justifies a future clinical trial to compare a resuscitation strategy with duration of cardiopulmonary resuscitation at providers' discretion before termination of resuscitation (a usual care group) versus a resuscitation strategy with prespecified duration of cardiopulmonary resuscitation before termination of resuscitation (an intervention group) for patients with in-hospital cardiac arrest.

Extracorporeal cardiopulmonary resuscitation is an advanced rescue therapy to support circulation in selected patients with refractory cardiac arrest, by an implantation of venoarterial extracorporeal membrane oxygenation.[34] A recent meta-analysis showed that extracorporeal cardiopulmonary resuscitation for patients with in-hospital cardiac arrest was associated with lower in-hospital morality.[35] As our study results showed that the probabilities of favorable outcomes decreased as duration of cardiopulmonary resuscitation increased, future research is needed to assess the optimal timing and patient selection to start extracorporeal cardiopulmonary resuscitation when cardiac arrest is refractory to conventional cardiopulmonary resuscitation in hospitals where extracorporeal cardiopulmonary resuscitation is available.

Strengths and limitations of study

Using the largest in-hospital cardiac arrest dataset in the world, we explicitly examined and quantified the changes in the probabilities of favorable outcomes for both patients undergoing cardiopulmonary resuscitation pending the first return of spontaneous circulation at a given moment and those who have achieved the first return of spontaneous circulation before or at the time point.

Our study has several limitations. Firstly, we used two definitions for the denominator of time dependent probability of survival and favorable

functional outcome among patients pending the first return of spontaneous circulation at each time point. The denominator that included patients who were undergoing cardiopulmonary resuscitation and patients who had termination of resuscitation relied on the assumption that all termination of resuscitation was appropriate. However, proving this assumption is not possible. In the denominator excluding those with termination of resuscitation, outcomes of patients who had termination of resuscitation without return of spontaneous circulation were censored. As duration of cardiopulmonary resuscitation is associated with patients' outcomes, the decision to terminate resuscitative efforts were likely affected by duration of cardiopulmonary resuscitation (self-fulfilling prophecy). Secondly, collecting time variables during cardiopulmonary resuscitation is difficult, and the precision of the collected time variables is an important limitation. However, the use of a large in-hospital cardiac arrest registry with standardized data definitions and data collection systems was intended to minimize this limitation. Thirdly, we were unable to account for the severity of underlying pre-arrest comorbidities, which could have been one of factors when termination of resuscitation was decided. However, we did multiple stratified analyses by clinical features and patient phenotypes to account for the potential confounders. Fourthly, unmeasured quality of cardiopulmonary resuscitation (for example, chest compression metrics) or post-resuscitation care might be different across treating teams, and such factors might be correlated with duration of cardiopulmonary resuscitation and patients' outcomes. Lastly, the generalizability of our findings is an important limitation as the GWTG-R is a voluntary registry and participation in the registry may reflect the interest in quality improvement of resuscitation of each hospital. Conversely, our findings are pertinent for programs intending to improve their resuscitation performance.

Conclusions

In this analysis of a large multicenter prospective registry of in-hospital cardiac arrest between 2000 and 2021 in the United States, we quantified the time dependent probabilities of survival to hospital discharge and favorable functional outcome at hospital discharge as a function of duration of cardiopulmonary resuscitation. The findings provide resuscitation teams, patients, and their surrogates with the objective probabilities of subsequent favorable outcomes if patients pending the first return of spontaneous circulation received further cardiopulmonary resuscitation.

What is already known on this topic

Longer duration of resuscitation for patients with in-hospital cardiac arrest is associated with decreased likelihood of survival

What this study adds

The time dependent probabilities of two outcomes among patients pending the first return of spontaneous circulation were quantified in each minute of cardiopulmonary resuscitation (CPR) duration

The outcomes assessed were survival to hospital discharge and favorable functional outcome at hospital discharge

The time dependent probabilities of survival and favorable functional status rapidly declined and were less than 1% at 39 minutes and at 32 minutes of CPR duration, respectively

Ethics statements

Ethical approval

This analysis of de-identified data was deemed exempt from regulations related to human subject research by the institutional review board at University of Pittsburgh (STUDY19020194).

Data availability statement

No additional data available.

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Footnotes

Contributors: MO and SK contributed to the design and conduct of the study, data collection and management, and analysis of the data. MO drafted the manuscript. All authors contributed to the interpretation of the data and presentation, review, and critical revision and approval of the manuscript. MO is the guarantor. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

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Competing interests:

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/disclosure-of-interest/ and declare: no support from any organization for the submitted work other than that described above; no financial relationships with any organizations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Transparency statement:

The lead author (the manuscript's guarantor) affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

Dissemination to participants and related patient and public communities:

The research findings will be disseminated through press releases, interviews with local and national media, social media posts on Twitter /X, and academic conferences.

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Sibling species of the major malaria vector Anopheles gambiae display divergent preferences for aquatic breeding sites in southern Nigeria

Faith I. Ebhodaghe, Irma Sanchez-Vargas, Clement Isaac, Brian D. Foy & Elizabeth Hemming-Schroeder

Abstract

Background

When integrated with insecticide-treated bed nets, larval control of *Anopheles* mosquitoes could fast-track reductions in the incidence of human malaria. However, larval control interventions may deliver suboptimal outcomes where the preferred breeding places of mosquito vectors are not well known. This study investigated the breeding habitat choices of *Anopheles* mosquitoes in southern Nigeria. The objective was to identify priority sites for mosquito larval management in selected urban and periurban locations where malaria remains a public health burden.

Methods

Mosquito larvae were collected in urban and periurban water bodies during the wet-dry season interface in Edo, Delta, and Anambra States. Field-collected larvae were identified based on PCR gel-electrophoresis and amplicon sequencing, while the associations between *Anopheles* larvae and the properties and locations of water bodies were assessed using a range of statistical methods.

Results

Mosquito breeding sites were either man-made (72.09%) or natural (27.91%) and mostly drainages (48.84%) and puddles (25.58%). *Anopheles* larvae occurred in drainages, puddles, stream margins, and a concrete well, and were absent in drums, buckets, car tires, and a water-holding iron pan, all of which contained culicine larvae. Wild-caught *Anopheles* larvae comprised *Anopheles coluzzii* (80.51%), *Anopheles gambiae* sensu stricto (*s.s.*) (11.54%), and *Anopheles arabiensis* (7.95%); a species-specific PCR confirmed the absence of the invasive urban malaria vector *Anopheles stephensi* among field-collected larvae. *Anopheles arabiensis, An. coluzzii*, and *An. gambiae s.s.* displayed preferences for turbid, lowland, and partially sunlit water bodies, respectively. Furthermore, *An. arabiensis* preferred breeding sites located outside 500 m of households, whereas *An. gambiae s.s.* and *An. coluzzii* had increased detection odds in sites within 500 m of households. *Anopheles gambiae s.s.* and *An. coluzzii* were also more likely to be present in natural water bodies; meanwhile, 96.77% of An. arabiensis were in man-made water bodies. Intraspecific genetic variations were little in the dominant vector *An. coluzzii,* while breeding habitat choices of populations made no statistically significant contributions to these variations.

Conclusion

Sibling malaria vectors in the *An. gambiae* complex display divergent preferences for aquatic breeding habitats in southern Nigeria. The findings are relevant for planning targeted larval control of *An. coluzzii* whose increasing evolutionary adaptations to urban ecologies are driving the proliferation of the mosquito, and *An. arabiensis* whose adults typically evade the effects of treated bed nets due to exophilic tendencies.

Background

Malaria remains a major public health challenge, with a disproportionately high burden of infections in Africa; ~ 95% of infection cases and ~ 96% of associated deaths are reported in the continent annually [1]. *Anopheles gambiae* sensu lato (*s.l.*) are the primary vectors of malaria in sub-Saharan Africa. These mosquitoes breed in clean and natural aquatic environments in the form of small sunlit water collections. However, deviations from this traditionally and widely known choice of breeding habitats by *An. gambiae s.l.* have been observed [2,3,] but these deviations remain understudied in countries in the West African sub-region. Furthermore, *An. gambiae s.l.* is a complex of mosquitoes comprising more than eight sibling species [4]. The anthropophilic and indoor-biting Afrotropical vectors *An. coluzzii* and *An. gambiae* sensu stricto (*s.s.*) —previously known as *M* and *S* molecular forms of *An. gambiae*, respectively— are sibling members of this complex and contribute to the high risk of malaria in Nigeria and neighbouring West and Central African countries [5].

Different mosquito larval surveys in West and Central Africa observed variations in the breeding habitat choices of *An. coluzzii* and *An. gambiae s.s.* (reviewed in [6]). In Burkina Faso, *An. coluzzii* co-existed with *An. gambiae s.s.* but preferred to breed in large, permanent, and vegetation-dense habitats (rice paddies), whereas *An. gambiae s.s.* preferred temporary puddles [7]. Additional differences in the ecologies of *An. coluzzii* and *An. gambiae s.s.* have been described in Mali [8] and Cameroon [9] among countries in the West and Central African sub-regions. Furthermore, evidence has

emerged supporting the hypothesis that contrasting responses of larvae to breeding habitat conditions formed the basis for ecological speciation of *An. coluzzii* and *An. gambiae s.s.* [10]. However, underlying ecological factors that underpin oviposition site preferences of gravid females and the water properties that mediate segregation of the breeding habitats of *An. coluzzii* and *An. gambiae s.s.*, are less well known. Addressing this knowledge gap, especially where *An. coluzzii* and *An. gambiae s.s.* are sympatric, is essential to reliably predict spatial distribution of larvae of the vector species and identify potential sites for targeted and species-specific mosquito larval control interventions.

Mosquito larval control interventions are effective for the control of malaria vectors. They also simultaneously target *Anopheles* and culicine disease vectors where these mosquitoes co-breed in water bodies [11]. Larval control interventions leverage biopesticides or predators to reduce the number of immature mosquitoes in aquatic environments and, where possible, may eliminate water bodies providing breeding places for mosquitoes [12]. However, mosquito larval control requires a clear understanding of the breeding habitats of target vectors in order to accurately select priority sites for interventions. Meanwhile, the World Health Organization [12] recommends mosquito larval control as a suitable method for supplementing pyrethroid-treated bed nets. This is because larval control reduces the abundance of pyrethroid-resistant mosquitoes, as well as outdoor-biting malaria vectors (e.g. *Anopheles arabiensis*) whose adults are typically outside the reach of pyrethroid-treated bed nets currently widely used in sub-Saharan African countries to control indoor-biting vectors.

Nigeria in West Africa reports the highest malaria disease burden worldwide, with > 25% of the global incidence of infections occurring in the country [1]. There are few reports on malaria spread by outdoor-biting mosquito species in Nigeria [13]. However, the typically indoor-biting vectors *An. coluzzii* and *An. gambiae s.s.* have been found to feed on humans in outdoor locations [14]. Some investigators attribute this to a behavioural response by vectors to the protracted use of pyrethroid-treated bed nets [15,16]. Long-term adoption of treated bed nets has increased the frequencies of pyrethroid-resistant vectors in wild mosquito populations in southern Nigeria [17,18], thus further compromising the efficacy of treated bed nets for malaria vector control. Although alternative intervention strategies, for example, mosquito larval control, are available for the management of malaria vectors, these strategies have received limited attention in southern Nigeria mainly due to the relatively low economic costs of using pyrethroid-treated bed nets.

Mosquito larval control interventions to manage pyrethroid- resistant *An. coluzzii* and *An. gambiae s.s.* could contribute to malaria risk reduction in southern Nigeria. These interventions could also assist in alleviating the epidemiologic burden of outdoor-biting *Anopheles* vectors that may be locally endemic but evading the effects of pyrethroid-treated bed nets. This study assessed the species diversity of *Anopheles* malaria vectors in selected urban and periurban areas in southern Nigeria. It further assessed vectors for differences in the choice of breeding habitats. Water bodies were surveyed for the presence and abundance of larvae and their physico-chemical properties characterised. Findings from the study add to current knowledge on the larval ecology of *An. gambiae s.l.* malaria vectors in Africa and provide relevant data for community-led and species-specific larval control interventions in urban and periurban settings in southern Nigeria where malaria risks are currently high and escalating.

Methods

Study area

Mosquito larval samplings were done in southern Nigeria with sites spread over a geographical distance of ≈200 km extending from Edo State (6° 17' 1.341" N, 5° 33' 59.061" E) to Delta State (6° 12' 6.523" N, 6° 10' 47.316" E) and Anambra State (6° 7' 9.12" N, 6° 47' 15.792" E). The human population size in these three neighboring States is ≈16 million [19]. According to the WHO Africa [19], household parasite screening surveys in 2021 based on the Rapid Diagnostic Technique (RDT) in children under 5 years of age indicated malaria infection rates of 30.2%, 18.9%, and 20.2% in Edo, Delta, and Anambra, respectively. Human exposure to infections is high in these areas during the wet season (May to mid-October), compared to the dry season (mid-October to April) when the numbers of vector breeding habitats are fewer. The average annual rainfall amount and temperature in southern Nigeria are 2500 mm and 27 ? , respectively, while vegetation is typically rainforest with extensive networks of freshwater swamps, and sparse and scattered woodlands [20].

Mosquito larvae sampling

Larval samplings were done during the late wet season and the early dry season from September to November 2022. To collect mosquito larvae in urban and periurban water bodies, a standard dipper (300 ml, John W. Hock's Company, Gainesville, Florida, USA) was lowered towards a water body and carefully but quickly applied to scoop the water surface. Where present in a water sample, mosquito larvae were morphologically identified as either *Anopheles* or culicine, counted, and stored in alcohol within small, labeled vials. To estimate average larval abundance, the overall number of larvae collected in a water body was divided by the total number of dips made in the same water body.

Characterization of water bodies

Water bodies within 500 m of households were considered 'close' while those outside 500 m were considered 'far'. A water body was 'turbid' if it was difficult to clearly see through water sample and 'non-turbid' if otherwise. To determine the

depth of mosquito larval sites, a straight pole was inserted in vertical position into a water body until the pole reached the bottom. Careful notice was made of the water-mark on the pole after it had been removed from water, while a graduated tape was used to measure the pole from the water mark down to the tip that touched the bottom of water body. Water bodies were considered 'deep' if they had a depth of above 20 cm and 'shallow' if depths were below 20 cm. A handheld GPS device (Garmin etrex 10) was used to record geographic coordinates and altitude of sampling sites. Water samples were assessed for 'temperature', 'pH', and 'salinity' at each site using a calibrated multi-parametric device (Hanna instrument GroLine Meter) powered by lithium batteries. Measurements of altitude (metres above sea level), temperature (?), pH, and salinity (parts per million) were considered high if they exceeded the 65th percentile values of their respective distributions, otherwise they were low. The 65th percentile values for altitude, temperature, pH, and salinity were 136 m (62 m to 316 m, SD: ± 57.46), 30.03 °C (22.7 ? to 36.1 ? , SD: ± 2.76), 7.74 (6.61 to 9.04, SD: ± 0.76), and 140 ppm (0 ppm to 410 ppm, SD: ± 101.79), respectively. Additional data collected at mosquito larval sites were the area (residential or industrial), site location (urban or periurban), habitat type (man-made or natural), vegetation presence (yes or no), presence of debris (yes or no), and water exposure to sunlight (partial or complete).

Molecular identification of *Anopheles* larvae

Genomic DNA was extracted from each individual *Anopheles* larva following the Chelex protocol described by Musapa et al. [21]. The Polymerase Chain Reaction (PCR) gel electrophoresis method was used to identify *Anopheles* larvae and to differentiate species of *An. gambiae s.l.* by targeting the *S200 X6.1* insertion polymorphism present in *An. coluzzii* but absent in *An. gambiae s.s.*, adopting the primers described by Santolamazza et al. [22] (Fwd: TCGCCTTAGACCTTGCGTTA and Rev: CGCTTCAAGAATTCGAG ATAC). PCR was conducted in 12.5 µl reaction volume containing 1 µl template DNA, 0.25 µl (10 µM) of each primer, 4.75 µl nuclease-free water, and 6.25 µl OneTaq® Quick-Load® 2X Master Mix (New England Biolabs).

PCR for *Anopheles* DNA amplification targeting the *S200 X6.1* gene was carried out on a Thermal cycler (Eppendorf Mastercycler nexus gradient) at 94 ? for 30 s; 30 cycles of denaturation at 94 ? for 15 s, 54 ? for 30 s and 68 ? for 1 min, and a final extension at 68 ? for 5 min. *Anopheles* larvae were identified based on a base-pair size of ~ 479 *An. coluzzii* (*M* form) and ~ 249 for *An. gambiae s.s.* (*S* form). The species *An. gambiae s.s.* has similar base-pair sizes with its siblings namely *An. arabiensis*, *An. melas*, and *An. quadriannulatus*.

DNA amplifications of the ITS_2 gene were carried out to identify samples that failed to amplify the S200 X6.1 gene using the primers ITS2A (Fwd: TGTGAACTGCAGG ACACAT) and ITS2B (Rev: TATGCTTAAATTCAGGGGGT), with reaction volume as described for the *S200 X6.1* PCR above. PCR adopted reaction conditions similar to those described previously [23]. PCR cycling was carried out on a Thermal cycler (Eppendorf Master- cycler nexus gradient) at 95 ? for 3 min; 35 cycles of denaturation at 94 ? for 30 s, 55 ? for 30 s, and 72 ? for 45 s, and a final extension at 72 ? for 6 min. Band size for Anopheles using the ITS_2 gene marker was ~ 750 bp.

In an attempt to identify samples whose DNA failed to amplify in *S200 X6.1*-PCR and ITS_2-PCR, an additional set of primers was used in endpoint-PCR namely, *St-F (CGTATCTTTCCTCGC ATCCA*) targeting a region of the ITS_2 gene specific to *An. stephensi* and the universal primers *U5.8S-F (ATCACTC GGCTCATGGATCG*) and *UD_2-R (GCAC TATCAAGCAACACGACT*) [24]. PCR was carried out in 12.5 µl reaction volume containing 1 µl template DNA, 0.25 µl (10 µM) of each of the primers *St-F* and *U5.8S-F* and 0.4 µl (10 µM) of the primer *UD_2-R*, 4.4 µl nuclease-free water, and 6.20 µl OneTaq® Quick-Load® $_2$X Master Mix (New England Biolabs). Cycling was carried out on a Thermal cycler (Eppendorf Mastercycler nexus gradient) at 95 ? for 30 s; 30 cycles of denaturation at 95 ? for 30 s, 55 ? for 30 s, and 68 ? for 45 s, and a final extension at 68 ? for 7 min. Band size for *An. stephensi* was ~438 bp with an internal control band of ~ 900 bp.

Nucleotide sequencing and phylogenetic analysis

The species identities of *Anopheles* were confirmed by unidirectional sequencing at Azenta Life Sciences, Colorado State University, USA. Sequencing was carried out on cleaned PCR-products (Exo-CIP™, New England Biolabs) using the *S200 X6.1* primer sequence TCGC CTTAGACCTTGCGTTA [22] and the *ITS_2B* primer sequence TATGCTTAAATTCAGGGGGT [23]. DNA sequences were visually inspected for quality in the *BioEdit* software [25]. Good-quality sequences were queried in BLAST analyses on the NCBI website [26]. Notes were taken of the sequence identities of query sequences compared to sequences of closest match in the GenBank. Clustal Omega [27] was used to align study and GenBank sequences, while the Smart Model Selection criterion in *PhyML* [28] was used to infer the best model of sequence evolution (Hasegawa-Kishino-Yano, HKY) [29]. Maximum-Likelihood phylogenetic trees were constructed in the software *Molecular Evolution and Genetic Analysis MEGA-X* [30], and the nodal support values of trees were estimated from 1000 bootstrap replications. Finally, genetic analyses to determine haplotype diversity (*Hd*) and polymorphic site number of *An. coluzzii* populations were carried out in *DnaSP* [31], while haplotype analyses were carried out using Median-Joining (MJ) networks [32]. MJ networks were constructed in the *PopART* software [33] with the aim to visualize relationships between populations of *An. coluzzii* larvae collected from water bodies in different geographical locations and having different properties.

Data analyses

The numbers of: (i) water body sites with mosquito larvae, (ii) geo-referenced locations surveyed, and (iii) mosquito larval collections were expressed in percentage frequencies with 95% confidence intervals (CI). Differences between percentage frequencies were assessed using a two proportion Z-test.

To determine their relative importance, predictors of larvae presence in aquatic environments were ranked based on Random Forest (RF) classification analyses. RF analyses were based on 10,000 iterations (ntrees) with 4 variables randomly selected at each split (mtry = $\sqrt{q}$ where q = the total number of variables (= 14)). The *R* functions '*importance* ()' and '*varImpPlot* ()' both embedded in the *randomForest* package version 4.7–1.1 [34] were used to generate Mean Decrease Gini (MDG) scores and variable importance plots, respectively. Variables with higher MDG scores were more important predictors of *Anopheles* larval presence in water bodies.

Generalized Linear Models (GLM) were used to assess associations between larvae and categorical predictor variables. Generalized Linear Models were fitted assuming a binomial distribution if response variable was binomial (*"larvae_yes"* or *"larvae_no"*), a negative binomial distribution if response variable was count (number of larvae per dip), and a quasi-binomial distribution if response variable was proportion (the number of larvae identified for a species divided by the total number of larvae analysed). A GLM assuming a binomial distribution was also used to assess the associations between *An. coluzzii* haplotypes and populations.

Variations in average larvae abundance were assessed using the Mann–Whitney *U* test. Correlations between larvae abundance and the following continuous variables (i) altitude, (ii) temperature, (iii) salinity, (iv) culicine abundance, and (v) pH were assessed in Spearman correlation tests and Principal Components Analysis (PCA). A Multiple Correspondence Analysis (MCA) was carried out to visually explore associations between larvae and water properties, as well as between larvae and location of mosquito breeding sites. PCA and MCA biplots were designed using the *R* packages '*FactorMineR*' and '*factorextra*' [35].

Multivariate Regression Models were fitted to account for possible confounding effects of variables. Predictor variables were selected for multivariate regression if they had $P < 0.05$ in univariate models. Furthermore, the backward elimination method was adopted to select predictor variables for the final multivariate model assuming a binomial distribution for binomial response variables, negative binomial distribution for count response variables, and quasi-binomial distribution for proportion response variables. Fisher's Exact test was used to assess predictor variables for association. Associated predictor variables were mutually exclusive in the final model. Multivariate analyses were followed by pairwise comparisons with *Tukey's* adjustment using the function '*emmeans*' embedded in the '*emmeans*' package [36]. All analyses were carried out in the *R* Statistical environment [37] while *P* values were set at an *alpha* of 0.05.

Results

Mosquito breeding habitats

A total of 43 water bodies among aquatic environments surveyed contained mosquito larvae. These water bodies were spread across 22 geo-referenced locations in Edo, Delta, and Anambra States (Table 1). These sites ranged from man-made (72.09%) to natural (27.91%) aquatic environments and comprised drains (48.84%), puddles (25.58%), abandoned car tires (9.30%), buckets (4.65%), drums (4.65%), and stream margins (2.33%), as well as iron pan (2.33%) and concrete well (2.33%) (Figs. 1 and 2). A total of 1,778 larvae collected comprised 32.34% *Anopheles* and 67.66% culicine mosquitoes. Additional file 1 shows the number of water bodies (according to habitat type) that were positive for Anopheles larvae and those that were positive for culicine larvae. Meanwhile, Additional file 2 provides an account of water properties identified to predict Anopheles larvae in water body sites.

Anopheles species diversity

Sequencing and species identification

Overall, 528 out of the 575 field-collected *Anopheles* larvae were analyzed in PCR, with success of DNA amplification for 382 larvae in *S200 X6.1* gene-PCR and 42 larvae in ITS_2-PCR; one larva was identified as *coluzzii-gambiae s.s.* hybrid in the *S200 X6.1* gene-PCR. The remaining 104 samples that failed to amplify in *S200 X6.1* gene-PCR and ITS_2-PCR were analysed using molecular markers that target amplification of *An. stephensi* DNA; however, none of these 104 samples amplified in endpoint-PCR except for the *An. stephensi* positive control included in the reaction. Further, 78 samples randomly selected from the 382 samples that amplified in *S200 X6.1* gene-PCR and all 42 samples that amplified in ITS_2-PCR were submitted to Sanger sequencing in order to confirm species identity. For *S200 X6.1* sequences, NCBI BLAST search identified 58 sequences as *An. coluzzii*, 4 sequences as *An. gambiae s.s.*, and 6 sequences as *An. arabiensis*, while 10 sequences had poor quality and were thus excluded from further analysis. For ITS_2 sequences, 7 sequences having poor quality were discarded, while 3 sample sequences were identified in NCBI BLAST analysis as *An. arabiensis* and 32 sample sequences were identified as *An. gambiae s.l.*

Agreements between PCR-gel electrophoresis and amplicon sequencing for Anopheles species identification

S200 X6.1 PCR and amplicon sequencing had near perfect agreement for the identification of *An. coluzzii* (Cohen's Kappa K = 0.84) and *An. gambiae s.s.* (Cohen's Kappa K = 0.90), but no agreement for the identification of *An. arabiensis* (Cohen's Kappa K = 0.00). The lack of agreement between *S200 X6.1* PCR and amplicon sequencing for *An. arabiensis* identification was due to the similarity

of band sizes between *An. gambiae ss* (~ 249 bp) and *An. arabiensis* (~ 223 bp). The similarity resulted in *An. arabiensis* mis-identification as *An. gambiae s.s.* in endpoint-PCR, but this mis-identification was corrected in amplicon sequencing. Six (6) *An. gambiae s.s.* samples and 2 *An. coluzzii* samples so identified by PCR were shown by sequencing to be *An. arabiensis* and *An. gambiae s.s.*, respectively. In an attempt to ensure that the study did not miss out on *An. arabiensis*, samples identified in endpoint PCR as *An. gambiae s.s.* were selected from different sites for amplicon sequencing.

Percentage identities and DNA sequence lengths Percentage identities of DNA sequences from the study when compared to GenBank DNA sequences ranged between 99.26% and 100% for the *S200 X6.1* sequences with base-pair (bp) lengths of between 171bp and 180bp for *An. arabiensis*, 194bp and 210bp for *An. gambiae s.s.*, and 407bp to 430bp for *An. coluzzii*. For ITS_2 sequences, percentage identities ranged between 99.5% and 100% with base-pair lengths of between 508bp and 516bp for *An. arabiensis* and 399bp and 531bp for *An. gambiae s.l.*

S200 X6.1 phylogeny Study DNA sequences of *An. coluzzii* on a maximum-likelihood phylogenetic tree (Fig. 3) clustered with GenBank DNA sequences of *An. coluzzii* whole genome (Accession No.: OX030893) and sequences of *An. gambiae* M molecular form from Mali (Accession No.: EU881869) and Nigeria (Accession No.: EU881872). Further, *An. gambiae s.s.* study sequences clustered with GenBank sequences of the whole genome of *An. gambiae s.s.* (Accession No.: OX030909) and a *An. gambiae s.s.* sequence from Senegal (Accession No.: EU881875). Lastly, DNA sequences of *An. arabiensis* from the study clustered with a sequence of the same species from Zimbabwe (Accession No.: Eu881886).

ITS_2 phylogeny On the maxi- mum-likelihood phylogenetic tree constructed from ITS_2 sequences (Fig. 3), *An. arabiensis* study sequences clustered with *An. arabiensis* GenBank sequences from Senegal (Accession Nos. MN335047, MN335048, and MN335040), Kenya (Accession No.: KJ522814) and Zambia (Accession No.: JN994133), whereas study sequences of *An. gambiae s.l.* clustered with GenBank sequences of *An. coluzzii* from Gabon (Accession No.: OL895513) and *An. gambiae* from Gabon (Accession No. OL895502) and Zambia (Accession No. Jn994138).

Anopheles coluzzii, An. gambiae s.s., and *An. arabiensis*

Overall distribution and proportions *An. coluzzii* occurred at more locations (68.18%, 15/22) compared to *An. arabiensis* (9.09%, 2/22) (Z-test: P = 0. 0002). However, the number of occurrence locations were similar between *An. coluzzii* and *An. gambiae s.s.* (36.36%, 8/22) (P = 0.07) and between *An. arabiensis* and *An. gambiae s.s.* (P = 0.07) (Fig. 4, Additional file 3). Overall, *An. coluzzii* larvae represented a greater proportion

Table 1 Geo-referenced locations of water bodies positive for mosquito larvae in urban and periurban areas in southern Nigeria (September to November 2022)

State	Location	Latitude	Longitude	Altitude	Mosquito larval habitat type
Edo	Aduwawa	6° 22' 21.96" N	5° 40' 24.78" E	106 m	Drain
	Ekenwan	6° 19' 23.52" N	5° 35' 49.92" E	87 m	Car tire, drain
	Ekiadolor*	6° 28' 45.72" N	5° 35' 2.46" E	136 m	Car tire, bucket, drum, iron pan
	GRA	6° 18' 18.3" N	5° 36' 18.18" E	66 m	Drain
	Ogbewase	6° 20' 6.12" N	5° 36' 39.9" E	132 m	Puddle
	Ogbeson	6° 20' 32.49" N	5° 41' 11.68" E	80 m	Drain, puddle
	Owina	6° 20' 7.14" N	5° 36' 19.08" E	94 m	Drain
	Sakponba	6° 18' 47.94" N	5° 38' 6" E	80 m	Car tire
	Ugbiyoko	6° 18' 51.48" N	5° 34' 10.68" E	77 m	Car tire, puddle
	Uwelu	6° 21' 44.04" N	5° 35' 59.05" E	92 m	Puddle
Delta	Agbor-Obi	6° 16' 2.52" N	6° 11' 8.94" E	131 m	Drain, drum
	Alihagwu*	6° 14' 52.2" N	6° 07' 53.82" E	168 m	Bucket
	Boji-Boji	6° 15' 56.1" N	6° 11' 35.58" E	116 m	Concrete well, drain, puddle
	Idumuoza*	6° 15' 47.40" N	6° 08' 21.72" E	169 m	Puddle
	Owa-Alero*	6° 12' 32.46" N	6° 13' 20.64" E	127 m	Puddle
	Owa-Eke*	6° 14' 4.44" N	6° 12' 52.02" E	203 m	Drain
	Umunede	6° 16' 20.16" N	6° 18' 11.52" E	252 m	Drain, puddle
Anambra	Ibolo-Oraifite*	6° 01' 20.34" N	6° 49' 2.64" E	62 m	Drain
	Nkpor*	6° 07' 9.9" N	6° 51' 56.28" E	113 m	Drain
	Nkwelle-Ezunaka*	6° 12' 26.94" N	6° 49' 50.88" E	77 m	Stream margin
	Odekpe*	6° 05' 13.8" N	6° 45' 15.9" E	64 m	Puddle
	Onitsha	6° 08' 55.86" N	6° 48' 21.3" E	110 m	Drain

*Peri-Urban

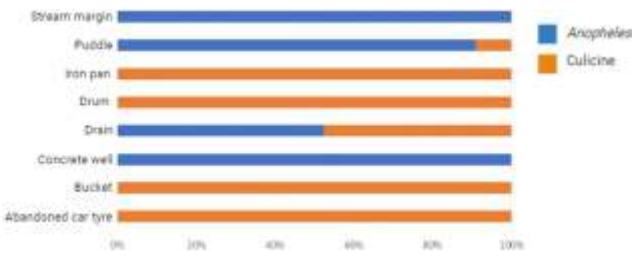
Fig. 1

Mosquito breeding sites inspected for *Anopheles* larvae in southern Nigeria: A Plastic bucket, B concrete well, C drainage, D iron pan, E Aluminum bucket, F stream margin, G puddle, and H drum

Fig. 3

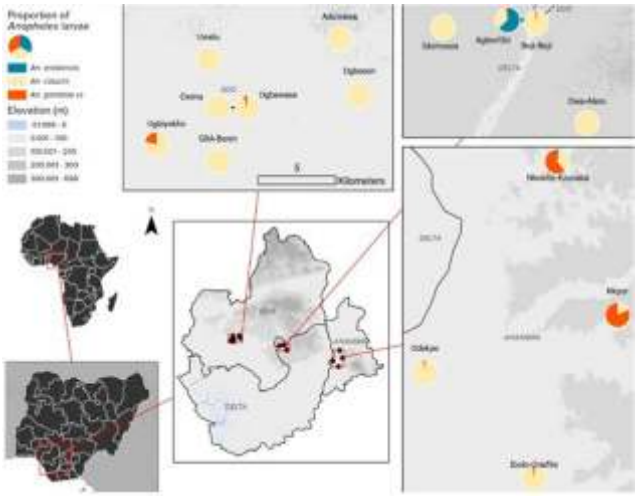
S200x6.1 DNA-based (left) and ITS2 DNA-based (right) Maximum-Likelihood phylogenetic trees. Each tree shows the phylogenetic relationships between *Anopheles* sample collected in southern Nigeria (September to November 2022). DNA sequences from this study end with the name of the state (Edo, Delta, or Anambra) in southern Nigeria where samples were collected, while sequences from GenBank are shown in bold. GenBank sequence of *An. merus* a nd *An. rivulorrum* have been selected as outgroup respectively for the *S200x6.1* DNA-based phylogeny (left) and ITS2 DNA-based phylogeny (right). Nodal support values based on 1000 bootstrap replicates are indicated next to the relevant nodes. The branch length represents substitution per site

Fig. 2



Proportions of *Anopheles* and culicine larvae in mosquito breeding sites in urban and periurban areas in southern Nigeria (September to November 2022)

Fig. 4



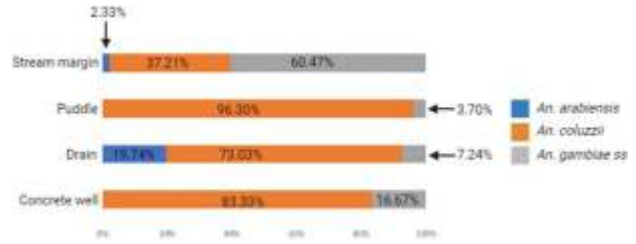
Map of sampling locations in southern Nigeria showing the relative proportions of *An. gambiae sl* larvae

(80.51%, 314/ 390) in comparison to *An. gambiae s.s.* (11.54%, 45/390) (Z-test: $P < 0.0001$) and *An. arabiensis* (7.95%, 31/390) ($P < 0.0001$), whereas *An. gambiae s.s.* and *An. arabiensis* occurred at similar proportions (P = 0.1165). *Anopheles coluzzii* were detected mainly in puddles (57.96%, 182/314), and then in drains (35.35%, 111/314), stream margin (5.10%, 16/314), and a concrete well (1.596%, 5/314), while for *An. gambiae s.s.*, detections were mainly in stream margin (57.78%, 26/45), followed by drains (24.44%, 11/45), puddles (15.56%, 7/45), and a concrete well (2.22%, 1/45) (Fig. 5). *Anopheles arabiensis* were detected at stream margin (3.23%, 1/31) and mainly in drains (96.77%, 30/31). *Anopheles coluzzii* and *An. gambiae s.s.* co-existed at 4 puddle sites and 3 drain sites, as well as in a concrete well. Mean- while, *An. coluzzii* co-existed with *An. arabiensis* in drains at 2 sites, whereas all three species co-existed at stream margin.

Multiple correspondence analysis The first two dimensions of the MCA explained 45.8% of variations in the properties of larval micro-habitats (Fig. 6). Twenty-five percent and 20.7% of these variations were respectively accounted for by dimensions 1 and 2. Sibling species of *An. gambiae s.l.* separated more clearly along dimension 2 than dimension 1. Among water properties, water turbidity and the presence of debris in water made greater contributions to variations on dimension 2. The vector *An. gambiae s.s.* occupied the negative axis of dimension 2, whereas *An. coluzzii* and *An. arabiensis* occupied the positive axis of the same dimension (Fig. 6).

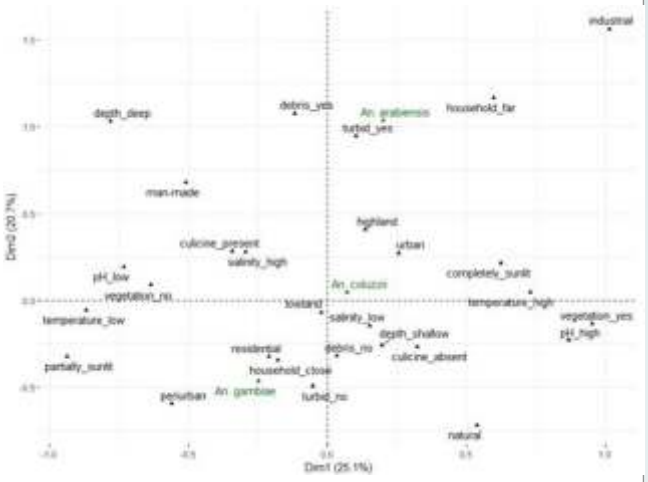
Separation of sibling species on the MCA biplot was more apparent for *An. gambiae s.s.* and *An. arabiensis* than for any other pair of the sibling vectors (Fig. 6). On dimension 2 of the

Fig. 5



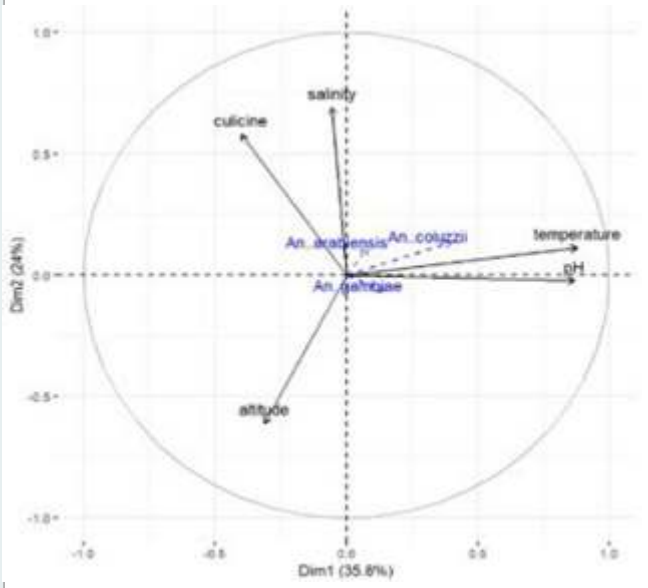
Relative proportions of sibling species of *An. gambiae sl* in different breeding habitat types in southern Nigeria

Fig. 6



A multiple correspondences analysis (MCA) biplot to visually illustrate the association between sibling malaria vectors and water properties as well as locations of mosquito breeding sites in southern Nigeria (September to November 2022)

Fig. 7



A principal component analysis (PCA) biplot to visually illustrate the association of sibling malaria vectors with topographic altitude and water properties of mosquito breeding sites

biplot and more than the other vectors, *An. gambiae s.s.* showed close association with non-turbid water bodies and aquatic environments without debris and with partial exposure to sunlight, while also having closer association with human dwellings and periurban locations. Whereas the association of *An. coluzzii* with water properties on dimension 2 was less clear, *An. arabiensis* displayed close associations with turbid water, as well as water bodies containing debris and those far from households.

Random Forest classification

On variable importance plots (Additional file 4), presence of culicine mosquitoes and debris, and habitat type and altitude were stronger predictors of *Anopheles* larvae and *An. coluzzii* presence (RF Accuracy 72.09%), whereas presence of *An. gambiae s.s.* depended more on turbidity, exposure to sunlight, and presence of culicine mosquitoes (RF Accuracy 83.72%) and *An. arabiensis* depended on culicine presence, temperature, and location (residential vs industrial) (RF Accuracy 93.02%).

Odds ratio analysis

The odds of *An. coluzzii* detection were greater in natural habitats (OR: 17.42, 95% CI 2.85, 339.61) and water bodies without debris (OR: 5.40, 95% CI 1.49, 22.22). These odds were also greater in lowlands (OR: 4.74, 95% CI 1.25, 21.13) and in aquatic environments with relatively high temperatures (OR: 6.18, 95% CI 1.55, 31.95) and pH (OR: 5.19, 95% CI 1.30, 26.80) (Table 2).

Odds of *An. gambiae s.s.* detection were greater in water bodies with partial rather than complete exposure to sunlight (OR: 6.50, 95% CI 1.40, 36.90), as well as in natural habitats (OR: 4.82, 95% CI 1.03, 24.60) and water bodies without culicine mosquitoes (OR: 5.83, 95% CI 1.22, 30.75) (Table 2).

Based on multivariate binomial regression modeling, topographic altitude and habitat exposure to sunlight respectively predicted the presence of *An. coluzzii* and *An. gambiae s.s.* in water bodies (Additional file 5). Habitat type also predicted the presence of *An coluzzii* and *An. gambiae s.s.* in the multivariate model analysis. The presence of *An. arabiensis* in water bodies was not assessed in multivariate regression due to small sample size.

Mean proportions

The relative mean proportion of *An. coluzzii* was greater in natural habitats (64.76, 95% CI 45.24, 84.27) and debris-free water bodies (46.96, 95% CI 31.58, 62.35) (Table 3). *Anopheles gambiae s.s.* also occurred at greater proportion in debris-free water bodies (6.05, 95% CI 0.45, 11.65), as well as in

Table 2 The odds ratio of various predictor variables for the sibling species *An. coluzzii, An. gambiae ss*, and *An. arabiensis* collected in urban and periurban areas in southern Nigeria (September to November 2022)

Predictor variables		Odds ratio (95% CI) (*P*-value, binomial GLM)					
		An. coluzzii	*P* value	*An. gambiae ss*	*P* value	*An. arabiensis*	*P* value
Culicine presence	Yes	NA	NA	0.14 (0.04, 0.36)	0.029	NA	NA
	No			5.83 (1.22, 30.75)			
Household	Far	0.63 (0.19, 1.87)	0.2	0.08 (0.00, 0.42)	0.19	0.18 (0.03, 0.68)	0.19
	Close	2.40 (0.64, 9.68)		4.36 (0.68, 85.97)		0.19 (0.01, 2.17)	
Area	Industrial	0.67 (0.17, 2.33)	0.33	NA	NA	0.25 (0.04, 1.00)	0.11
	Residential	2.04 (0.49, 9.29)				0.13 (0.01, 1.46)	
Location	Periurban	1.00 (0.31, 3.20)	0.78	0.50 (0.13, 1.59)	0.22	0.09 (0.00, 0.47)	0.83
	Urban	1.21 (0.31, 4.72)		0.38 (0.08, 1.87)		0.76 (0.07, 17.29)	
Altitude	Highland	0.40 (0.11, 1.20)	0.028	0.08 (0.00, 0.39)	0.15	NA	NA
	Lowland	4.75 (1.25, 21.13)		4.95 (0.78, 97.40)			
Habitat	Man-made	0.63 (0.30, 1.29)	0.0099	0.15 (0.04, 0.38)	0.047	0.07 (0.01, 0.23)	0.83
	Natural	17.42 (2.85, 339.61)		4.82 (1.03, 24.60)		1.32 (0.06, 15.16)	
Turbidity	Yes	1.00 (0.39, 2.56)	0.7	0.06 (0.00, 0.29)	.062	0.13 (0.02, 0.44)	0.39
	No	1.27 (0.38, 4.36)		8.00 (1.27, 156.90)		0.33 (0.01, 3.76)	
Debris	Yes	0.42 (0.13, 1.12)	0.013	0.06 (0.00, 0.31)	0.078	0.13 (0.02, 0.47)	0.34
	No	5.40 (1.49, 22.22)		7.11 (1.12, 139.48)		0.30 (0.01, 3.39)	
Vegetation	No	0.87 (0.41, 1.82)	0.21	0.27 (0.10, 0.63)	0.91	0.08 (0.01, 0.26)	0.95
	Yes	2.31 (0.64, 9.09)		0.92 (0.17, 4.17)		0.93 (0.04, 10.54)	
Depth	Deep	1.00 (0.24, 4.23)	0.83	0.33 (0.05, 1.45)	0.75	0.14 (0.01, 0.80)	0.51
	Shallow	1.19 (0.24, 5.77)		0.75 (0.14, 5.86)		0.42 (0.04, 9.87)	
pH	Low	0.71 (0.33, 1.47)	0.029	0.21 (0.07, 0.50)	0.4	0.07 (0.01, 0.25)	0.98
	High	5.19 (1.30, 26.80)		1.92 (0.40, 8.82)		1.04 (0.05, 11.83)	
Salinity	Low	1.00 (0.49, 2.06)	0.49	0.25 (0.09, 0.57)	0.82	0.07 (0.01, 0.24)	0.9
	High	1.60 (0.43, 6.38)		1.20 (0.22, 5.57)		1.17 (0.05, 13.35)	
Temperature	Low	0.65 (0.29, 1.37)	0.016	0.17 (0.05, 0.43)	0.15	0.04 (0.00, 0.17)	0.26
	High	6.18 (1.55, 31.95)		3.00 (0.66, 14.50)		4.15 (0.37, 94.32)	
Exposure to sunlight	Complete	1.23 (0.59, 2.60	0.75	0.12 (0.03, 0.33)	0.022	0.07 (0.01, 0.25)	0.98
	Partial	0.81 (0.22, 2.95)		6.50 (1.40, 36.90)		1.04 (0.05, 11.83)	

NA Not Available due to small sample size. *P* values <0.05 are shown in bold.

water bodies that are non-turbid (5.93, 95% CI 0.13, 11.73) and partially sunlit (9.50, 95% CI 0.00, 19.83) (Table 3).

Proportions of *An. coluzzii* were negatively associated with altitude (ρ = ? 0.33, P = 0.033) and positively associated with temperature (ρ = 0.36, P = 0.019). Figure 7 shows the direction and magnitude of these associations. Among predictor variables, only habitat exposure to sunlight could predict the proportions of *An. gambiae s.s.* in multivariate quasibinomial regression models (Additional file 5). No predictor variable was identified to statistically predict the proportions of *An. coluzzii* in multivariate models. The association between predictor variables and proportions of *An. arabiensis* could not be assessed in multivariate regression due to the small sample size.

Haplotype diversity of *An. coluzzii* populations based on DNA sequences of the *S200 X6.1* gene

A total of 58 *An. coluzzii* DNA sequences (391 bp) were analysed to assess possible effects of breeding habitat choices on genetic variations in An. coluzzii populations. The DNA sequences of *An. coluzzii* consisted of 6 haplotypes (H1 to H6), with a diversity (Hd) of 0.62 (Additional file 6). These haplotypes segregated at 5 polymorphic sites, in addition to one nucleotide deletion in one of the study sequences (GenBank Accession Number: OR700036).

Additional file 7 shows the nucleotide positions of these segregations with reference to the *An. coluzzii* Ngousso genome hosted in VectorBase [38]. The nucleotide substitution A > G was the most common mutation while the nucleotide substitution A > C was the least common mutation. The H1 haplotype was more likely to occur in urban locations (OR: 3.80, 95% CI 1.21, 13.62) than periurban locations (OR:

Table 3 The proportions of sibling malaria vectors in mosquito breeding sites in urban and periurban areas in southern Nigeria (September to November 2022)

Predictor variables		Mean proportion (95% CI) (P-value, Mann–Whitney U test)					
		An. coluzzii	*P* value	*An. gambiae ss*	*P* value	*An. arabiensis*	*P* value
Culicine abundance	High	22.41 (1.53, 43.28)	0.116	4.86 (0.00, 13.09)	0.519	NA	
	Low	40.95 (26.14, 55.77)		3.14 (0.00, 6.52)		6.34 (0.00, 15.28)	
Household	Close	41.84 (27.65, 56.04)	0.1357	3.48 (0.20, 6.76)	0.227	0.05 (0.00, 0.17)	0.151
	Far	17.49 (0.00, 39.12)		4.33 (0.00, 13.75)		13.54 (0.00, 33.50)	
Area	Residential	38.42 (24.99, 51.85)	0.3473	4.87 (0.44, 9.30)		0.05 (0.00, 0.15)	0.064
	Industrial	21.49 (0.00, 50.28)	NA			17.60 (0.00, 44.13)	
Location	Urban	35.14 (21.27, 49.00)	0.9772	1.84 (0.10, 3.57)	0.216	5.68 (0.00, 13.74)	0.902
	Periurban	32.80 (5.88, 59.71)		8.65 (0.00, 20.91)		0.14 (0.00, 0.44)	
Altitude	Lowland	39.32 (25.11, 53.53)	0.1293	5.11 (0.10, 10.12)	0.135	6.12 (0.00, 14.75)	
	Highland	24.46 (0.97, 47.95)		0.89 (0.00, 2.82)		NA	
Habitat	Natural	64.76 (45.24, 84.27)	0.001	6.00 (0.00, 13.96)	0.062	0.14 (0.00, 044)	0.902
	Man-made	22.76 (9.88, 35.65)		2.86 (0.00, 6.74)			
Turbidity	Non-turbid	38.31 (21.88, 54.74)	0.5079	5.93 (0.13, 11.73)	0.041	0.07 (0.00, 0.20)	0.358
	Turbid	29.16 (10.57, 47.76)		0.69 (0.00, 2.16)		9.78 (0.00, 23.92)	
Debris	No	46.96 (31.58, 62.35)	0.012	6.05 (0.45, 11.65)	0.049	0.06 (0.00, 0.19)	0.311
	Yes	15.40 (0.00, 31.73)		0.20 (0.00, 0.61)		10.35 (0.00, 25.37)	
Vegetation	Yes	47.37 (24.19, 70.55)	0.104	1.85 (0.00, 4.36)	0.844	5.90 (0.00, 18.55)	1.000
	No	27.58 (13.76, 41.40)		4.75 (0.00, 9.92)		3.18 (0.00, 9.59)	
Depth	Shallow	35.10 (21.80, 48.41)	0.869	3.76 (0.00, 7.86)	0.709	2.57 (0.00, 7.71)	0.525
	Deep	31.77 (0.00, 65.79)		3.65 (0.00, 9.37)		10.94 (0.00, 36.80)	
pH	High	50.01 (29.77, 70.25)	0.071	2.10 (0.00, 4.78)	0.572	6.32 (0.00, 19.97)	0.953
	Low	26.99 (12.31, 41.66)		4.53 (0.00, 9.53)		3.07 (0.00, 9.25)	
Salinity	High	40.98 (16.56, 65.40)	0.494	1.66 (0.00, 4.45)	0.956	6.80 (0.00, 21.63)	0.881
	Low	31.67 (17.41, 45.92)		4.64 (0.00, 9.46)		2.97 (0.00, 8.93)	
Temperature	High	46.52 (25.30, 67.75)	0.053	5.71 (0.00, 13.84)	0.191	11.73 (0.00, 28.87)	0.226
	Low	28.03 (13.34, 42.72)		2.68 (0.00, 6.07)		0.06 (0.00, 0.18)	
Exposure to sunlight	Partial	32.28 (9.28, 55.28)	0.848	9.50 (0.00, 19.83)	0.013	0.12 (0.00, 0.37)	1.000
	Complete	35.55 (20.83, 50.27)		0.96 (0.00, 2.23)		6.07 (0.00, 14.70)	

NA Not Available due to small sample size. *P* values <0.05 are shown in bold.

0.29, 95% CI 0.10, 0.74) (P = 0.028). Aside this, no other association was detected between haplotype and geographical location or property of water bodies. Further analysis revealed low genetic variations between An. coluzzii populations that selected different breeding sites (Additional file 8).

Discussion

This study characterized the species diversity and breeding habitat choices of Anopheles malaria vectors in selected urban and periurban areas in southern Nigeria. Wild-caught Anopheles mosquito larvae comprised *An. coluzzii, An. gambiae s.s.*, and *An. arabiensis* with preferred breeding sites in lowland, partially sunlit, and turbid water bodies, respectively. Furthermore, *An. coluzzii* and *An. gambiae s.s.* showed close association with breeding sites within 500 m of households, whereas *An. arabiensis* were associated with breeding sites outside 500 m of households.

Similar to findings in Ghana's Cape Coast in West Africa [2], *An. coluzzii* occurred over a wider spatial range and at higher proportions compared to other sibling species. This may be explained by the adaptation of *An. coluzzii* larvae to predator pressure in the wild and the vector's ability to outcompete sibling species in natural environments [39]. Most mosquito breeding grounds in southern Nigeria had dried up during the dry season campaign. This left behind permanent breeding grounds that favour the proliferation of *An. coluzzii* and, as in a previous work [7], contributed to increase collection of this vector species.

The overexpression of detoxification genes by *An. coluzzii* has been demonstrated to enable larval individuals to exploit polluted urban breeding sites in Central Africa [40] and may further explain the high odds of *An. coluzzii* in urban and periurban water bodies in southern Nigeria. Findings revealed that *An. coluzzii* larvae were more likely to occur in water bodies in lowlands than in highlands. Except for

a few cases where broad flat surfaces provided breeding places for mosquitoes in highlands [41], water bodies are more stable for mosquito breeding activities in lowlands. Also, warm temperatures in lowlands are favourable for mosquito larval survival and assist to accelerate rates of larval development [42,43,44].

Even though sampling did not include adult mosquitoes, it is likely that *An. coluzzii* dominated adult populations of malaria vectors during the predominantly dry sampling period in southern Nigeria and that this contributed to increase collections of *An. coluzzii* larvae in study locations. Interestingly, earlier studies in similar ecologies that were conducted during dry periods of the year when temperatures are high alluded to the dominance of *An. coluzzii* among adult Anopheles mosquitoes [45, 46]. Under high temperature conditions, adult *An. coluzzii* are more able than other sibling vectors to minimize water loss and they do this by a variety of methods including altering the chemical compositions of cuticular hydrocarbons [47].

Anopheles gambiae s.s. have the behaviour of ovipositing in temporary, rain-dependent, small water collections such as puddles and hoof-prints [6]. As evaporation of small water collections is likely to occur over shorter periods during dry seasons, it seems that *An. gambiae s.s.* in southern Nigeria have developed a strategy that allows more time to complete development of immature stages before breeding habitats completely dry up. This strategy, as the study results suggest, involves *An. gambiae s.s.* preference for water collections that are partially rather than completely exposed to sunlight. The present study therefore hypothesizes that direct and complete exposure to sunlight could hasten evaporation of temporary water bodies where *An. gambiae s.s.* breed and result in the death of immature mosquitoes before they reach adulthood.

For multiple reasons, wild-caught larvae in Delta and Anambra were not expected to include the outdoor-biting mosquito *An. arabiensis*. Firstly, *An. arabiensis* typically inhabit arid savannah landscapes and are often absent in field collections of adult or larval mosquitoes in the humid rainforest zone of southern Nigeria [48]. Secondly, except on very few occasions [49,50,51], past and recent surveys in Delta and Anambra States have failed to detect *An. arabiensis* in field campaigns [14,17,52]. Possible reasons for the absence of *An. arabiensis* in these earlier campaigns include but are not limited to the possibility that the vector was simply not present in sampling areas, or that investigators focused samplings on adult mosquitoes indoors and identified mosquitoes using less-sensitive techniques that are incapable of teasing apart sibling species.

In separate studies in Burkina Faso in West Africa [7] and Kenya in East Africa [53], An. arabiensis had its highest abundance during the dry season in October about the same time when *An. gambiae s.s.* had its lowest abundance. Mosquito larval collection during a similar period of the year likely increased the chances of *An. arabiensis* detection. This mosquito species is zoophilic and occurs close to livestock [54], thus it is not surprising that mosquito sampling led to collection of many *An. arabiensis* larvae in water bodies in Agbor-Obi (Delta State) where there are several pockets of livestock-keeping areas.

The association between *An. arabiensis* and livestock production has been confirmed in a plethora of studies in sub-Saharan Africa [55,56,57] and is based on the fact that female mosquitoes, usually, restrict flight activities to places near animal blood meal hosts and oviposit in water bodies nearby. This could also explain why *An. coluzzii* and *An. gambiae s.s.*, being anthropophilic mosquitoes, had almost all their breeding sites close to human residence within 500 m of households. In the Suba District in Kenya, households where > 90% of adult *An. gambiae s.s.* were collected also had larval sites within 300 m [58]. By breeding near blood meal hosts, female mosquitoes conserve flight energy and enable young adult progenies to easily access blood meals shortly after emergence.

Contrary to their choice of clean water bodies (see review by [48,57], *An. arabiensis* larvae occurred in polluted water in drainages in Agbor-Obi. *Anopheles arabiensis* larvae had also been found in polluted urban drainages and irrigation canals in the Khartoum State of Sudan [59]. Furthermore, in Bobo-Dioulasso (Burkina Faso) where *An. arabiensis* has adapted to breeding in the polluted Houet river and can therefore transmit malaria throughout the dry season [60,61], the vector species increased in composition among malaria vectors from 3 to 90% [60] over a period of two decades. Detections of *An. arabiensis* in these types of breeding places continue to increase [62,63], signaling a persistent and continuous adaptation of *An. arabiensis* larvae to polluted water bodies in urban areas, thereby promoting malaria transmission throughout the dry season.

In Central Africa, larvae of *An. arabiensis* that developed in organic wastewater developed faster, resulting in adults that had longer longevity and larger phenotypic sizes, as well as increased resistance to insecticides [64]. Data on the association between An. arabiensis choice of breeding habitats and insecticide resistance traits are currently sparse in southern Nigeria. However, preliminary results from ongoing insecticide resistance studies in Agbor-Obi indicate the presence of the pyrethroid-resistant mutation L995F in larvae of *An. arabiensis* from polluted aquatic environments [unpublished data]. However, investigators are yet to find positive cases of L995F in *An. arabiensis* from clean water pools along stream margins in Nkwelle-Ezunaka (Anambra State).

In line with findings from the survey in southern Nigeria, a study in central Ethiopia identified *An. arabiensis* in turbid water collections [65]. Similar observations of malaria vector preference for turbid water bodies were made in Tanzania [66]. However, the species identity of mosquitoes was not determined. *Anopheles* mosquitoes typically avoid turbid for clean water for the reason that suspended insoluble particles interfere with larvae ingestion of food materials [65]. These particles also limit sunlight penetra- tion of water and

consequently, slow down the production of aquatic microphyte food materials for mosquito larvae. These may have been responsible for the avoidance of turbid water bodies by *An. gambiae s.s.* and *An. coluzzii* in southern Nigeria.

However, turbidity may have less effect on *An. arabiensis* where larvae have adequate access to food materials. In the Ye-Ebiyo et al. [65] study in central Ethiopia, *An. arabiensis* were unaffected by turbidity of water bodies but only when larval sites were close to flowering maize plants providing pollen grains for larvae nourishment. In the present study, turbid water bodies that contained *An. arabiensis* larvae also contained organic debris. As Jeanrenaud et al. [64] observed in Cameroon, these organic wastes probably served as food for *An. arabiensis* larvae in southern Nigeria.

The Asian urban malaria vector *An. stephensi* invaded Africa in 2012 [67] and has since been expanding its spatial range in the continent [68], with the most recent detections of the vector made in the West Africa sub-region [51,69], first in 2020 in Gombe in northern Nigeria [51]. Due to its potential to drive malaria outbreaks [67,70], *An. stephensi* surveillance has received increased attention in areas of potential invasion. Molecular analysis in the present study screened *Anopheles* larval samples for *An. stephensi* because some samples could not be identified by molecular markers used in *An. gambiae* identification; similar experiences of molecular markers failing to identify wild samples of *Anopheles* mosquitoes led to the first reports of *An. stephensi* in different locations in Africa [71]. Moreover, Sinka and colleagues [72] identified the study area in southern Nigeria among places in West Africa where ecological conditions are favourable for the invasion and establishment of *An. stephensi*.

Furthermore, given *An. stephensi zoophagic* habits [73], it is possible that the frequent pastoralists' movement of livestock from Gombe and neighboring locations in northern Nigeria to grazing fields and slaughterhouses in southern Nigeria could provide a route for and facilitate the southward spread of *An. stephensi*. In this study, mosquito larval samplings were carried out during the wet-dry season interface at a time when *An. stephensi* occur in high abundance [72] and with a majority of larval sampling sites comprising man-made water containers where the vector species prefers to breed in urban locations [11]. Considering that the larval sampling strategy maximized opportunities for *An. stephensi* detection, the non-report of the vector among the study mosquito samples therefore suggests its absence in the sampling area and likely slow spread in the country Nigeria. However, *An. stephensi* possesses potential for rapid spatial distribution. This has been demonstrated in East Africa, where the vector species was detected in five countries within a period of 10 years [68] and in West Africa, where it was recently detected in Accra Ghana [69], just less than 3 years after the initial detection in northern Nigeria [51].

The *An. coluzzii* population in southern Nigeria was moderately genetically diverse, with a haplotype diversity index of 0.62. This suggests that vector control interventions are currently not optimally effective at reducing *An. coluzzii* abundance in the study area; otherwise, vector populations would have presented with low genetic diversity. High genetic diversity of *An. coluzzii* was attributed to variations in the ecology of larval development sites along the Gambian River in West Africa [74]. In southern Nigeria, *An. coluzzii* that developed in periurban larval sites had slightly more haplotypes than those in urban sites. However, and possibly as an indication of *An. coluzzii's* attempt to adapt to otherwise less favorable conditions in urban ecological landscapes, one of the two dominant haplotypes detected in *An. coluzzii* occurred in close association with the urban vector population. Due to small sample size, the use of a less informative molecular marker, and the restriction of nucleotide sequencing to limited regions of the genome, it was difficult to adequately assess genetic divergence between *An. coluzzii* populations in the present study.

Turbidity metres or Secchi disks are recommended for reliable assessment of water turbidity; hence investigators admit that the method of assessing water turbidity based on physical observations may have been less accurate. Physical observation to assess water turbidity could also be subjective; however, in the present study, water turbidity assessment by the same person helped to address this challenge in the field. Further, the study did not systematically evaluate the effects of biological factors. Some of these biological factors, for example the presence of predators, have been shown in previous studies to affect *Anopheles* larvae in aquatic environments (reviewed in [6,75]. However, the relative proportions of *Anopheles* larvae and the fact that sibling vectors rarely co-existed in water body sites suggest that sibling mosquitoes could be engaging in some sort of inter- specific competition for resources [39,76]). The stream margin in Nkwelle-Ezunaka (Anambra) was the only place where *An. arabiensis*, *An. gambiae s.s.* and *An. coluzzii* occurred together in a single water body. It could be that mosquito larvae, because they are less crowded in large breeding sites such as stream margins, are less likely to engage in resource competition. Still on mosquito interactions, Anopheles and culicine larvae had inverse associations in southern Nigeria. The inverse association observed between Anopheles and culicine larvae corroborates the principle that gravid dipterans typically avoid breeding places already exploited by conspecific and heterospecific females [77]. This behaviour has been reported in *An. gambiae s.s.* [78] and aims is to ensure adequate food resources for potential immature progenies and thus enhance the biological fitness of adult progenies.[12]

In conclusion, the study reports different breeding habitat choices for three sibling malaria vectors in southern Nigeria. The dominant vector *An. coluzzii* prefer breeding sites in lowlands while *An. gambiae s.s.* prefer sites that are partially rather than completely exposed to sunlight. In contrast to *An. arabiensis* that display association with man-made sites outside 500 m of households, *An.*

coluzzii and *An. gambiae s.s.* have a high likelihood to breed in natural sites within 500 m of households. These findings suggest that *An. coluzzii* and *An. gambiae s.s.* are more likely than *An. arabiensis* to infect humans in residential places where the vectors co-exist [79]. And as they are typically indoor feeders, An. *coluzzii* and *An. gambiae s.s.* have a greater chance of contacting and being killed by treated bed nets.

There are ongoing efforts by State governments to upscale the distribution and encourage the use of pyrethroid-treated bed nets for malaria vector control in southern Nigeria [19]. However, *An. arabiensis*, being an outdoor feeder and capable of deriving bloodmeals from multiple vertebrates in addition to humans, has a lower opportunity of encountering treated bed nets. In East Africa, treated bed nets helped control *An. gambiae s.s.*, but *An. arabiensis* were only slightly affected, thus leaving behind a post-intervention phase of residual malaria transmission by *An. arabiensis* [80]. Findings of pyrethroid resistance mutations in *An. arabiensis* in the study area [unpublished data] and escalations of insecticide resistance in *An. coluzzii* in southern Nigeria [18] further dampen the prospect of vector control using treated bed nets.

Malaria control programmes in southern Nigeria could leverage findings from the present study in designing targeted larval control interventions. Across sub-Saharan Africa, larval control interventions have been explored to reduce the population abundance of *An. arabiensis* and a couple of other mosquito species that transmit infections, irrespective of vector biting location (indoors or outdoors) or insecticide resistance status (resistant or susceptible) [81,82]. Larval control is particularly useful in the context of southern Nigeria where bed net interventions are apparently having limited effects on malaria vectors. Larval control interventions to reduce human malaria transmission are easier to implement during the dry season when several water collections providing breeding places for *Anopheles* vectors have dried up [12]. As rainfall amounts decrease, mosquito breeding activities are concentrated to fewer water collections, which, if targeted in larval control interventions, could improve the goal of reducing malaria risks in periods of little or no rainfall. The present study has identified potential sites for larval control interventions during such periods in southern Nigeria. It has also reported the absence of *An. stephensi* in selected urban and periurban locations in the area. However, southern Nigeria is exposed to *An. stephensi* invasion being a travel destination for land, air, and sea transport from places where the vector species has already established its presence. This raises a need for the National Malaria Control Programme and relevant health authorities at the subnational levels to create a system for the surveillance of urban and periurban locations for *An. stephensi*. Such surveillance should focus on man-made mosquito breeding sites where larval interventions could help to slow the spread and proliferation of *An. stephensi* in the event of an invasion.

Availability of data and materials

All data generated and/or analysed during this study are included in this published article and in the supplementary files. The raw dataset will be made available upon reasonable request to the corresponding author. DNA sequences generated from the study have been deposited in the GenBank database using the Accession Numbers OR700033 to OR700102, and OR717035 to OR717056.

Abbreviations

BP: Base-pair
CI: Confidence interval
DNA: Deoxyribonucleic Acid
GLM: Generalized Linear Model
Hd : Haplotype diversity
ITS2: Internal Transcribed Spacer-2
MCA: Multiple Correspondence Analysis
MDG: Mean Decrease Gini
OR: Odds ratio
PCA: Principal Components Analysis
PCR: Polymerase chain reaction
RF: Random Forest
SINE: Short Interspersed Elements
? : Degree Celsius
WHO: World Health Organization
RDT: Rapid Diagnostic Test
mm: Millimeter
MJ: Median-Joining

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Contributions

FIE conceived the research. FIE, EHS, BDF, CI designed the research. EHS supervised the study. FIE collected field samples. FIE, ISV performed lab work. FIE analysed the data. FIE drafted the manuscript. FIE, EHS, ISV, BDF edited the manuscript. All authors read and approved the final version of the manuscript.

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Ethics declarations

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Competing interests

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Ab-Externo MicroShunt versus Trabeculectomy in Primary Open-Angle Glaucoma: Two-Year Results from a Randomized, Multicenter Study

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Purpose

To compare the effectiveness and safety of the MicroShunt (Santen Inc) versus trabeculectomy in patients with primary open-angle glaucoma (POAG).

Design

Prospective, randomized, multicenter trial conducted in the United States and Europe.

Participants

Adult patients (aged 40–85 years) with mild to severe POAG inadequately controlled on maximum tolerated medical therapy and intraocular pressure (IOP) ≥15 mmHg and ≤40 mmHg.

Methods

Patients were randomized 3:1 to stand-alone MicroShunt implantation (n = 395) or trabeculectomy (n = 132), both augmented with mitomycin C (MMC) 0.2 mg/ml for 2 minutes.

Main Outcome Measures

The primary effectiveness end point was surgical success, defined as ≥20% reduction in mean diurnal IOP from baseline with no increase in glaucoma medications. Secondary end points included changes in mean IOP and medication use from baseline and the need for post-operative interventions.

Results

At 2 years, the rate of surgical success was lower in the MicroShunt group than in the trabeculectomy group (50.6% vs. 64.4%, P = 0.005). Mean diurnal IOP was reduced from 21.1 ± 4.9 mmHg at baseline to 13.9 ± 3.9 mmHg at 24 months in the MicroShunt group and from 21.1 ± 5.0 mmHg at baseline to 10.7 ± 3.7 mmHg at 24 months in the trabeculectomy group (P < 0.001 compared with baseline in both groups). Mean medication use decreased from 3.1 to 0.9 in the MicroShunt group and from 2.9 to 0.4 in the trabeculectomy group (P < 0.001 compared with baseline in both groups). Adverse events at 2 years were generally similar in the 2 groups, except that hypotony was more common in eyes undergoing trabeculectomy (51.1% vs. 30.9%, P < 0.001). Repositioning or explantation of the implant occurred in 6.8% of MicroShunt patients. The majority of these patients had device removal at the time of subsequent glaucoma surgery. Vision-threatening complications were uncommon in both groups.

Conclusion

At 2 years, both the MicroShunt and trabeculectomy provided significant reductions in IOP and medication use, with trabeculectomy continuing to have greater surgical success.

Financial Disclosure(s)

Proprietary or commercial disclosure may be found in the Footnotes and Disclosures at the end of this article.

Keywords

Antifibrotic agents, Aqueous drainage devices Minimally invasive glaucoma surgery Open-angle glaucoma Trabeculectomy

Abbreviations and Acronyms

BCVA, best-corrected visual acuity, CI, confidence interval, IOP, intraocular pressure, MIGS, micro-invasive glaucoma surgery, MMC, mitomycin C, POAG, primary open-angle glaucoma

Since its description in 1968, the

Cairns-type trabeculectomy has been the clinical standard for surgical reduction of intraocular pressure (IOP) in eyes with glaucoma.[1] Based on its efficacy, trabeculectomy is the preferred procedure in eyes with advanced glaucoma or very low interventional IOP.[2,3,4,5] However, its safety profile —with potentially sight-threatening complications, including bleb leaks, choroidal effusions, hypotony maculopathy, and blebitis/endophthalmitis—may limit its value in eyes with more modest therapeutic goals.[4,6] Trabeculectomy also requires a high level of surgeon skill and experience to appropriately manage a delicate series of operative and postoperative manipulations and adjustments. The advent of a family of procedures collectively referred to as "micro-invasive glaucoma surgery" (MIGS) has expanded the indications for surgical intervention in eyes with glaucoma. The MIGS family includes procedures that shunt aqueous humor across the trabecular meshwork to Schlemm's canal or the suprachoroidal space. Some of these procedures require the implantation of a permanent device, whereas others are limited to the incision or excision of various ocular tissues.[7,8,9,10,11] In general, MIGS procedures have a more favorable safety profile compared with trabeculectomy, but with lower efficacy. Thus, canal-based MIGS procedures may be viable options for patients who would benefit from surgical IOP reduction but whose more modest therapeutic goals may not justify the risks of trabeculectomy.[7,8,9,10,11]

The MicroShunt (Santen Inc) is an implantable device, measuring 8.5 mm in length and 0.35 mm in width, with a lumen diameter of 70 µm and a pair of 0.375-mm fins for intrascleral fixation. The tube length and lumen diameter were selected to accommodate sloughed endothelial cells (40–50 µm) while providing sufficient resistance to limit pressure reduction and minimize the risk of hypotony.[12] The MicroShunt is implanted subconjunctivally via an ab-externo approach and provides filtration from the anterior chamber to the subconjunctival space without the need for scleral flap formation, sclerostomy, or iridectomy.

This 2-year trial compared the effectiveness and safety of the MicroShunt with trabeculectomy in patients with uncontrolled primary open-angle glaucoma (POAG), using maximal tolerated medical therapy, in the United States and Europe. Interim 1-year results of this trial have been reported.[13] The present study describes the 2-year results of this trial.

Methods

This was a prospective, single-masked, randomized, multicenter, noninferiority trial conducted at 24 sites in the United States and 5 sites in Europe. The study was conducted in accordance with the tenets of the Declaration of Helsinki, as well as all applicable local regulations.[14] The study protocol was approved by all relevant ethics boards, and all subjects provided written informed consent to participate. The trial was registered at ClinicalTrials.gov (NCT01881425).

The study design, eligibility criteria, treatments, and surgical details have been reported previously.[13] Briefly, this trial enrolled patients aged 40 to 85 years with mild to severe POAG (visual field mean deviation –3.0 decibels or worse) with IOP = 15 mmHg and = 40 mmHg, inadequately controlled on maximal tolerated medical therapy. Key exclusion criteria included secondary glaucoma, vision level of no light perception, prior ocular surgery involving the conjunctiva, and the requirement for general anesthesia for the study procedure. Only 1 eye was enrolled per patient. Eligible subjects were randomly assigned 3:1 to undergo MicroShunt implantation or trabeculectomy; unequal allocation was deemed applicable to this confirmatory trial, because it allowed recruitment of a sufficient number of eyes in the treatment arm to detect rare adverse events. Both procedures were augmented with mitomycin C (MMC) 0.2 mg/ml applied for 2 minutes via saturated sponges on bare sclera. For the MicroShunt procedure, a 1-mm double-step knife was used to fashion a narrow scleral track with a wider scleral pocket at its distal end. The device was threaded through the track and the fins tucked into the pocket. Flow of aqueous humor from the anterior chamber was confirmed, and Tenon's capsule and conjunctiva were sutured in watertight closure. Fornix-based trabeculectomy was performed in standard fashion with watertight closure of Tenon's capsule and conjunctiva.

Patients were evaluated at 1 day, 1 and 4 weeks, and 3, 6, 12, 18, and 24 months after surgery. Parameters measured included best-corrected visual acuity (BCVA), IOP by Goldmann tonometry (mean of 2 readings within 2 mmHg or 3 readings if the difference was > 2 mmHg), medication use, and complications. The trial protocol specified that the window for year 1 (12 month) visits was on study days 330 to 420 and the window for year 2 (24 month) visits was on study days 690 to 780. At baseline and months 12 and 24, diurnal IOP was assessed at 9 am, 12 pm, and 4 pm. Before randomization, an interventional IOP (above which an IOP-lowering intervention would

be applied) was set for each eye as either 21 mmHg or preoperative IOP if it was < 21 mmHg or as an IOP selected by the investigator based on individual risk factors. Interventions included laser suture lysis, needling, and laser disruption of outflow obstruction; medications were generally initiated only after unsuccessful attempt at these procedural interventions, unless such attempts were believed unlikely to achieve interventional IOP.

Efficacy was analyzed in the intent-to-treat population (all randomized subjects) with the baseline-observation-carried-forward approach to imputation of missing data for the primary effectiveness end point, defined as a = 20% reduction from baseline in mean diurnal IOP at the month 24 visit without increasing the number of preoperative IOP-lowering medications, consistent with guidelines set by the World Glaucoma Association.[15] End point failures included loss of light perception at 2 consecutive visits, IOP < 6 mmHg at 2 consecutive visits after month 3, incisional or laser reoperation (except for suture lysis, needling, or laser removal of outflow obstruction) for complications or inadequate IOP control, and the need for oral carbonic anhydrase inhibitors for IOP control. A Farrington-Manning test was used to construct 95% confidence intervals (CIs) around the difference in success rates between treatment groups. Farrington-Manning tests were performed to test for non-inferiority, and Wald-type tests were performed to test the equality of rates. Time to first failure was assessed using Kaplan–Meier survival analysis with treatments compared using the log-rank test. Prespecified subgroup analyses in patients with baseline mean diurnal IOP < 18, 18 to < 21, and = 21 mmHg were conducted.

Secondary effectiveness end points included mean diurnal IOP change at month 24 and the need for postoperative interventions by month 24. Mean diurnal IOP change was evaluated using a mixed-effects model for repeated measures with Hochberg step-up adjusted P values based on a 2.5 mmHg noninferiority margin (as in the Tube Versus Trabeculectomy study).[16] The mean number of glaucoma medications required at each visit was an exploratory efficacy end point.

The proportions of eyes in each group requiring interventions through month 24 and the incidence of specific qualifying reoperations (bleb revision, repositioning or removal of implant, resuturing scleral flap, trabeculectomy, glaucoma drainage device, and iridotomy/iridectomy) were compared using Wald-type 2-sample proportions tests for the equality of rates.

Safety was analyzed in the safety population, consisting of all subjects who underwent surgery. Prespecified safety end points included the occurrence of adverse events, development of cataract as assessed using the Lens Opacities Classification System, Version III in phakic eyes, time for postoperative BCVA to return to baseline, and change in endothelial cell density. Endothelial cell density was measured using a Konan specular microscope, with all measurements performed at the endothelial cell density Corneal Image Analysis Reading Center at the Department of Ophthalmology and Visual Sciences at Case Western Reserve University School of Medicine.[13]

Sample size calculations were based on a Z-test with normal distribution approximation and assuming an annual dropout rate of 6%.[13] A sample size of 514 patients was calculated for the primary effectiveness end point assuming a month 12 IOP success rate of 0.74, a 1-sided significance level of 0.025, 90% statistical power, and a noninferiority margin of 0.15 (15%).

Results

Subject Disposition and Demographics

The intent-to-treat population included 527 subjects who were randomized to treatment with the MicroShunt (n = 395) or trabeculectomy (n = 132) (Fig 1). Of these, all but 1 patient randomized to trabeculectomy (who required general anesthesia and was thus ineligible) underwent the assigned study procedure and comprised the safety population. By the end of the second year of the study, 9 subjects (7 in the MicroShunt and 2 in the trabeculectomy group) were lost to follow-up, and 25 subjects (18 in the MicroShunt group and 7 in the trabeculectomy group) discontinued the study. Of the 18 patients in the MicroShunt group who discontinued, 5 died, 3 discontinued due to adverse events, 8 withdrew consent, and 2 were withdrawn by the investigator. Of the 7 patients in the trabeculectomy group who discontinued, 1 died, 4 withdrew consent, 1 was withdrawn by the investigator, and 1 had been enrolled after closure of the study enrollment window. Small proportions of patients visited at times outside the prespecified follow-up times. Specifically, of the 527 randomized patients, only 11 (2.1%) visited outside the 12-month window of 330 to 420 days and only 8 (1.5%) visited outside the 24-month window of 690 to 780 days. The demographic and ocular characteristics of the study population are shown in Table 1. Baseline characteristics were similar in the 2 groups except for ethnicity, with a higher proportion

of Black subjects in the MicroShunt group than in the trabeculectomy group (18.0% vs. 8.3%, P = 0.04).

Primary Effectiveness End Point

Surgical success (≥20% reduction in IOP from baseline with no increase in the number of glaucoma medications) was observed in 50.6% of subjects in the MicroShunt group and 64.4% in the trabeculectomy group (P = 0.005) (Fig 2A). Subgroup analysis showed that at year 2, the between-group difference in surgical success in eyes with baseline IOP < 18 mmHg was –12.8% (95% CI, –29.5 to 4.0; P = 0.141; Fig 2B); in eyes with IOP ≥18 mmHg but < 21 mmHg, this difference was –22.2% (95% CI, –41.7 to –2.8, P = 0.012; Fig 2C); and in eyes with IOP ≥ 21 mmHg, this difference was –11.5% (95% CI, –26.4 to 3.3; P = 0.114; Fig 2D). The time to first failure in each group is shown in Figure 3. Median survival times could not be determined, because less than 50% of the eyes in each group had failed by study end. The nature of failures is shown in Table 2. Failure to achieve an IOP reduction of ≥20% from baseline was the most common reason for failure in the MicroShunt group, whereas persistent hypotony was the leading cause of failure in the trabeculectomy group.

Figure 2. Comparisons of surgical success rates at 24 months in the (A) overall MicroShunt (Santen Inc.) and trabeculectomy groups, and in subgroups of patients with baseline intraocular pressure (IOP) (B) < 18 mmHg, (C) 18 to < 21 mmHg, and (D) ≥21 mmHg. Success was defined as a ≥20% reduction in IOP from baseline with no increase in the number of glaucoma medications and no criteria for failure met. Results were determined on the basis of multiple imputation, with

Figure 1. Consolidated Standards of Reporting Trials (CONSORT) diagram showing the disposition of study subjects. ITT = intent-to-treat.

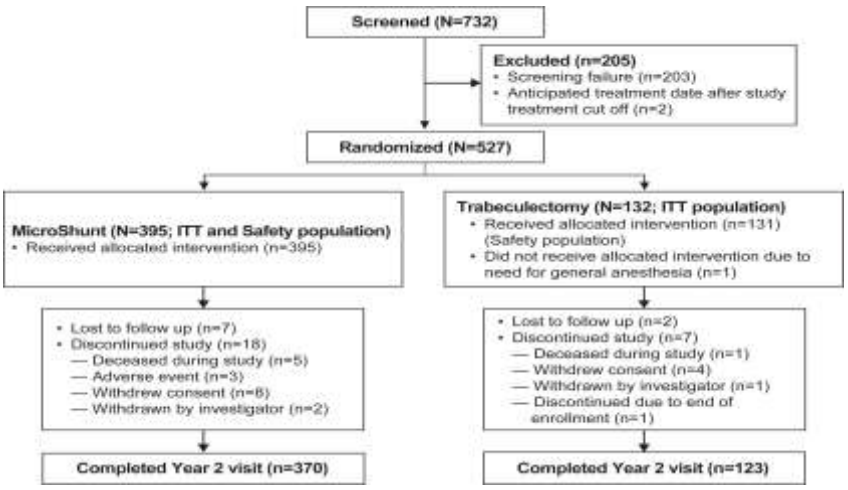


Table 1. Baseline Demographic and Ocular Characteristics of the Patients Included in the Study

	MicroShunt (Santen Inc.) Group (n = 395)	Trabeculectomy Group (n = 132)	*P* Value
Mean ± SD age, yrs	66.4 ± 9.3	67.8 ± 9.3	0.14
Male, n (%)	181 (45.8)	73 (55.3)	0.06
Race, n (%)			0.04
White	311 (78.7)	113 (85.6)	
Black/African American	71 (18.0)	11 (8.3)	
Asian	10 (2.5)	6 (4.5)	
Other	3 (0.8)	2 (1.5)	
Lens status, n (%)			0.26
Phakic	227 (57.5)	76 (57.6)	
Pseudophakic	168 (42.5)	56 (42.4)	
Baseline IOP, n (%)			
< 18 mmHg	119 (30.1)	45 (34.1)	0.40
≥18 and < 21 mmHg	116 (29.4)	31 (23.5)	
≥21 mmHg	160 (40.5)	56 (42.4)	
Mean ± SD IOP, mmHg	21.1 ± 4.9	21.1 ± 5.0	0.99
Mean ± SD number of glaucoma medications	3.1 ± 1.0	2.9 ± 0.9	0.31
Mean ± SD Humphrey VF MD, dB	–12.3 ± 7.0	–12.4 ± 7.1	0.88
Glaucoma severity classification, n (%)			0.37
Early (–3.00 dB to –6.00 dB)	84 (21.3)	30 (22.7)	
Moderate (–6.01 dB to –12.00 dB)	134 (33.9)	46 (34.8)	
Advanced (–12.01 dB to –20.00 dB)	119 (30.1)	33 (25.0)	
Severe (≤ 20.01 dB)	58 (14.7)	22 (16.7)	

dB = decibels; IOP = intraocular pressure; MD = mean deviation; SD = standard deviation; VF = visual field.

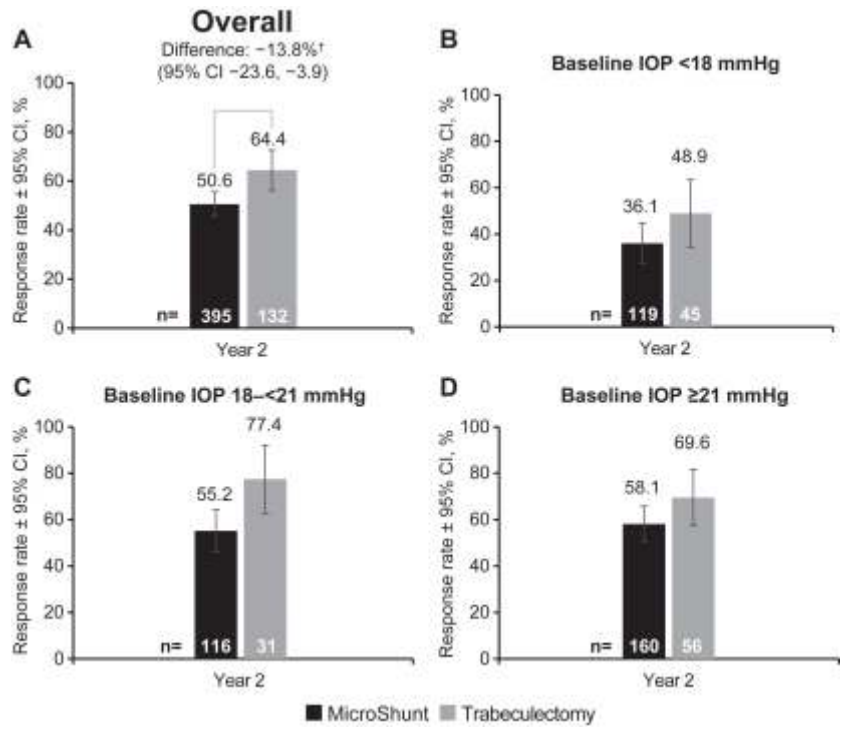
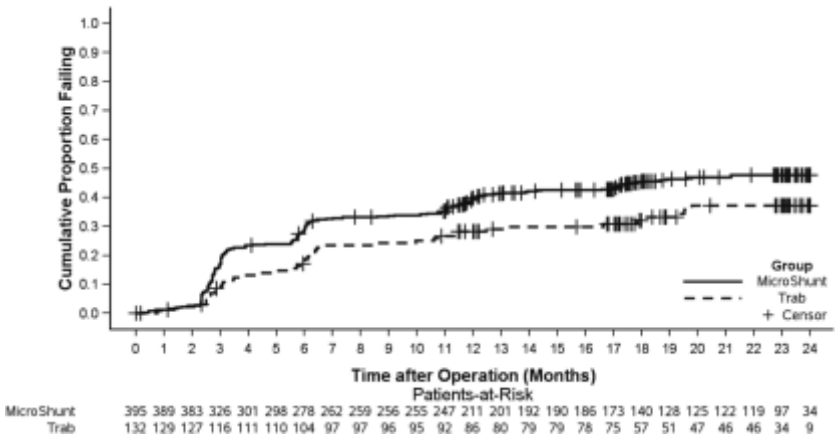


Figure 2. Comparisons of surgical success rates at 24 months in the (A) overall MicroShunt (Santen Inc.) and trabeculectomy groups, and in subgroups of patients with baseline intraocular pressure (IOP) (B) < 18 mmHg, (C) 18 to < 21 mmHg, and (D) ≥ 21 mmHg. Success was defined as a ≥ 20% reduction in IOP from baseline with no increase in the number of glaucoma medications and no criteria for failure met. Results were determined on the basis of multiple imputation, with P values calculated by 2-sample proportion Wald-type tests. CI = confidence interval.

Figure 3. Kaplan–Meier analysis of time to first failure in the MicroShunt and trabeculectomy groups through 2 years.



P values calculated by 2-sample proportion Wald-type tests. CI = confidence interval.

Secondary Effectiveness End Points

Mean IOP by treatment group at each study time point is shown in Figure 4. Mean IOP was higher in eyes in the trabeculectomy compared with the MicroShunt group during the first postoperative week, indicative of tight closure of the scleral flap and subsequent suture lysis after day 7. From month 3 onward, mean IOP was lower in the trabeculectomy group, by approximately 3 mmHg, at each time point through the end of the study. Mean IOP from month 3 to year 2 was approximately 14 mmHg in eyes in the MicroShunt group and approximately 11 mmHg in eyes in the trabeculectomy group.

Glaucoma medication use at baseline and months 12, 18, and 24 is shown in Figure 5A. Mean medication use was reduced from 3.1 ± 1.0 at baseline to 0.9 ± 1.3 at month 24 in MicroShunt-treated eyes and from 2.9 ± 0.9 to 0.4 ± 0.9 in trabeculectomy-treated eyes ($P < 0.001$ compared with baseline in both groups). The proportions of eyes that were medication-free at 12, 18, and 24 months are shown in Figure 5B. At month 24, 61.1% of eyes in the MicroShunt group and 79.8% of eyes in the trabeculectomy group were medication-free.

To assess regional differences, outcomes were compared in the United States and the European Union. The baseline demographic and clinical characteristics were well matched across groups by location and intervention (Table S3, available at www.aaojournal.org). Surgical success rates were higher in the European Union than in the United States (Fig S6, available at www.aaojournal.org). Patients in the United States who underwent

Table 2. Reasons for Surgical Failure at 2 Years in > 5% of Patients in the MicroShunt and Trabeculectomy Groups in the Intent-to-Treat Population

	MicroShunt Group (n = 395), n (%)	Trabeculectomy Group (n = 132), n (%)	*P* Value
IOP reduction < 20% from baseline	116 (29.4)	12 (9.1)	<0.001
IOP < 6 mmHg at 2 consecutive visits	15 (3.8)	20 (15.2)	<0.001
Qualifying reoperation*in the study eye	74 (18.7)	14 (10.6)	0.014
Other glaucoma surgery to reduce IOP	41 (10.4)	5 (3.8)	0.004

IOP = intraocular pressure.

All surgical failures are shown. A total of 45 subjects are listed in more than 1 category due to concurrent failures.

Qualifying reoperations include trabeculectomy, placement of a drainage device, bleb revision (other than needling), explantation or repositioning of the MicroShunt, iridotomy/iridectomy, or resuturing of the scleral flap.

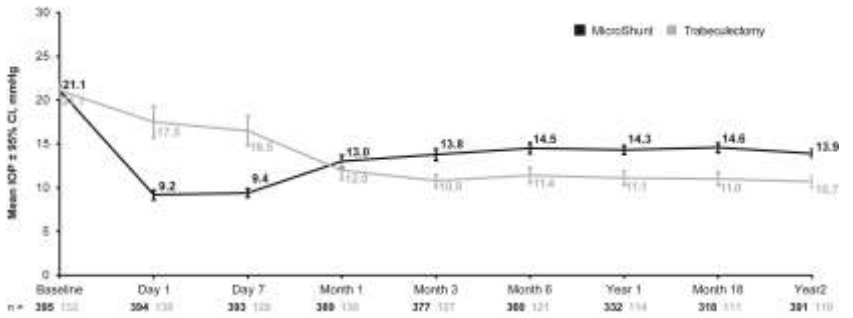


Figure 4. Mean intraocular pressure (IOP) in the MicroShunt and trabeculectomy groups at each study time point. Mean diurnal IOP was measured at baseline and Year 1 by taking standard IOP measurements at 9:00 AM ± 1.5 hours, 12:00 PM ± 1 hour, and 4:00 PM ± 2 hours. The 3 standard IOP measurements were used to determine the mean diurnal IOP; standard IOP was defined as the mean of 2 readings within 2 mmHg of each other or the median of 3 readings if > 2 mmHg apart; based on multiple imputation. Data presented for the ITT population. CI = confidence interval; ITT = intention-to-treat.

MicroShunt implantation and trabeculectomy had mean diurnal IOPs of 21.1 ± 5.1 mmHg and 21.2 ± 5.2 mmHg, respectively, at baseline, decreasing to 14.1 ± 4.1 mmHg and 10.6 ± 3.7 mmHg, respectively, at 2 years (P < 0.001 compared with baseline in both groups). The differences in mean changes from baseline were –6.4 ± 5.8 mmHg in the MicroShunt group and –10.1 ± 5.5 mmHg in the trabeculectomy group, with the between-group difference being statistically significant (P < 0.001).

In comparison, patients in the European Union who underwent MicroShunt implantation and trabeculectomy had mean diurnal IOPs of 20.6 ± 3.4 mmHg and 20.3 ± 3.7 mmHg, respectively, at baseline, decreasing to 13.3 ± 2.8 mmHg and 11.5 ± 3.7 mmHg, respectively, at 2 years (P < 0.001 compared with baseline in both groups). The differences in mean changes from baseline were –7.1 ± 3.6 mmHg in the MicroShunt group and –8.3 ± 5.4 mmHg in the trabeculectomy group, with the between-group difference not being statistically significant (P = 0.296). Within the United States, the percentages of patients who were medication-free were 60.8% in the MicroShunt group and 81.5% in the trabeculectomy group. In the European Union, the corresponding figures were 63.2% and 68.8%, respectively.

Patients with low baseline IOP (< 18 mmHg) were compared in the MicroShunt (n = 119) and trabeculectomy (n = 45) groups. The demographic and clinical characteristics of these 2 groups were similar (Table 4). Mean diurnal IOP ± standard deviation in the MicroShunt and trabeculectomy groups were 16.4 ± 0.9 mmHg and 16.6 ± 0.9 mmHg, respectively, at baseline, decreasing to 12.9 ± 3.2 mmHg and 10.6 ± 4.3 mmHg, respectively, at 2 years (P < 0.001

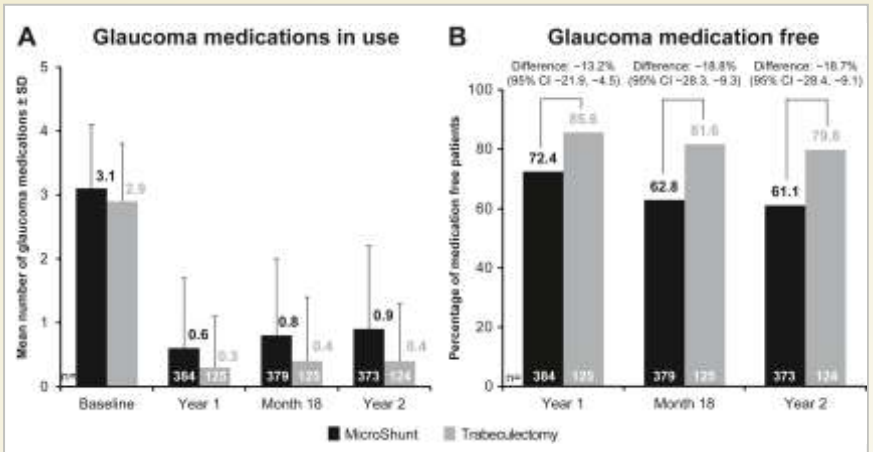


Figure 5. Medication use (A) and the proportions of medication-free eyes (B) at key time points in the MicroShunt and trabeculectomy groups. Data presented for the ITT population. CI = confidence interval; ITT = intention-to-treat; SD = standard deviation.

Table 5. Cumulative Postoperative Adverse Events and Other Safety Outcomes Occurring at Year 2 in > 5% of the Safety Population of the MicroShunt or Trabeculectomy Group

Outcome	MicroShunt Group (n = 395), n (%)	Trabeculectomy Group (n = 131), n (%)	*P* Value
Increased IOP requiring treatment	222 (56.2)	73 (55.7)	0.924
Hypotony (IOP <6 mmHg at any time)	122 (30.9)	67 (51.1)	<0.0001
Worsening of VF MD ≥2.5 dB	73 (18.5)	27 (20.6)	0.580
Loss of ≥2 lines of BCVA	56 (14.2)	23 (17.6)	0.369
Bleb leak	36 (9.1)	19 (14.5)	0.113
Corneal edema	35 (8.9)	8 (6.1)	0.277
Shallow anterior chamber	27 (6.8)	11 (8.4)	0.568
Diplopia	26 (6.6)	7 (5.3)	0.595
Choroidal effusion/ detachment	19 (4.8)	10 (7.6)	0.270
Cataract progression	56 (14.2)	28 (21.4)	0.071
Ptosis	33 (8.4)	7 (5.3)	0.211
Pain	26 (6.6)	13 (9.9)	0.248
Encapsulated bleb	23 (5.8)	3 (2.3)	0.045

BCVA = best-corrected visual acuity; dB = decibels; IOP = intraocular pressure; MD = mean deviation; VF = visual field.

compared with baseline in both groups), with 66.1% and 69.8%, respectively, being medication-free at 2 years. The composite primary end point of surgical success was achieved by 36.1% of patients in the MicroShunt group and 48.9% in the trabeculectomy group (non-inferiority P = 0.398). Rates of postoperative interventions and postoperative adverse events did not differ significantly in these 2 groups.

Safety

Table 5 shows the frequency and nature of adverse events, each of which occurred in > 5% of subjects in either group through 2 years. The most common adverse event in both the MicroShunt and trabeculectomy groups was increased IOP requiring treatment (56.2% vs. 55.7%; P = 0.924). Hypotony, defined as IOP < 6 mmHg at any time point, was significantly more frequent in the trabeculectomy group than in the MicroShunt group (51.1% vs. 30.9%; P < 0.001). Surgical failure due to hypotony (IOP < 6 mmHg at 2 consecutive visits) also occurred more often in the trabeculectomy group (15.2% vs. 3.8% P < 0.001). Hypotony maculopathy was detected in 0.5% of MicroShunt-treated and 1.5% of trabeculectomy-treated eyes. Endothelial cell density decreased significantly in both groups over 2 years (P < 0.001), with mean reductions of 7.7% in the MicroShunt group and 9.6% in the trabeculectomy group (P = 0.242) (Fig 7). No cases of endophthalmitis occurred in either group.

Mean BCVA was modestly reduced (by 5 ETDRS letters) at month 1 in both groups and returned to preoperative values (within 1–3 letters) by month 3 in both groups. Rates of lens opacity (Lens Opacities Classification System, Version III) in phakic eyes at 2 years, defined as postoperative lens opacity or

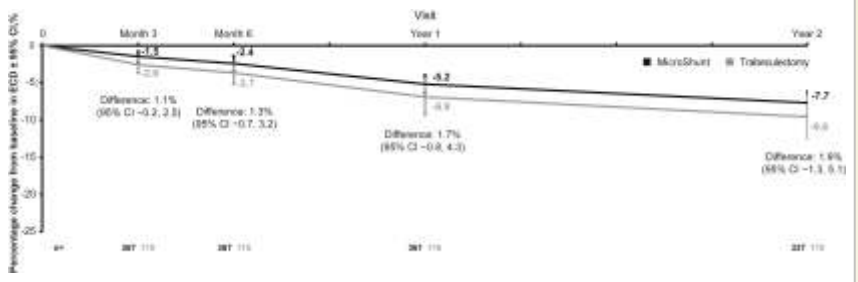


Figure 7. Percentage change in endothelial cell density. Data presented for the safety population. CI = confidence interval; ECD = endothelial cell density; SD = standard deviation.

Table 6. Postoperative Interventions, Including Qualifying Operations through 2 Years, in the MicroShunt and Trabeculectomy Groups

	MicroShunt Group (n = 395), n (%)	Trabeculectomy Group (n = 132), n (%)	*P* Value
Any postoperative intervention	219 (55.4)	93 (70.5)	<0.001
Qualifying reoperation in the study eye*	74 (18.7)	14 (10.6)	0.014
Bleb revision	40 (10.1)	10 (7.6)	0.3551
Placement of drainage device	19 (4.8)	4 (3.0)	
Removal of implant	19 (4.8)	0 (0.0)	
Trabeculectomy	9 (2.3)	1 (0.8)	
Repositioning of the MicroShunt	8 (2.0)	0 (0.0)	
Resuturing of the sclera flap	6 (1.5)	4 (3.0)	
Iridotomy/iridectomy	3 (0.8)	0 (0.0)	
Needling of bleb with or without injected antifibrotic	98 (24.8)	12 (9.1)	<0.001
Laser suture lysis before secondary trabeculectomy	0 (0.0)	69 (52.3)	<0.001
Introduction of glaucoma medication	201 (50.9)	52 (39.4)	0.020
Other†	18 (4.6)	2 (1.5)	0.042

P values based on 2-sample proportion test (Wald type).

Qualifying reoperations include trabeculectomy, placement of a drainage device, bleb revision (other than needling), explantation or repositioning of the MicroShunt, iridectomy, or resuturing of the scleral flap. P values are not calculated for all reoperation subcategories due to the low number of events.

† Including laser removal of blockage, laser suture lysis after any secondary trabeculectomy, glaucoma laser procedure, or use of viscoelastic to limit aqueous flow.

worsening of preexisting lens opacity, were similar in the MicroShunt (41.1% [78/190]) and trabeculectomy (47.6% [30/63]) groups (P = 0.361). Cataract surgery through month 24 was performed in 7.6% of eyes in the MicroShunt group and 13.0% of eyes in the trabeculectomy group (P = 0.095).

Through 2 years of follow-up, a higher percentage of MicroShunt-treated patients than trabeculectomy-treated patients required bleb needling with an antifibrotic agent (most commonly MMC) (24.8% vs. 9.1%; P < 0.001). Of these eyes that underwent needling, 21.4% and 25% from the MicroShunt and trabeculectomy groups, respectively, were needled more than once.

Qualifying reoperations for complications or inadequate IOP control (Table 6) were more common in the MicroShunt group than in the trabeculectomy group (18.7% vs. 10.6%; P = 0.014). Bleb revision with opening of the conjunctiva was the most common procedure in both groups (10.1% vs. 7.6%). Nineteen patients (4.8%) required MicroShunt explantation, including 4 for device-related reasons/complications. Of these 4 patients, 2 experienced anterior migration of the implant, requiring device removal due to endothelial concerns; 1 underwent explantation due to persistent hypotony, and 1 underwent explantation due to device-associated erosion of the conjunctiva. The other 15 patients underwent MicroShunt removal at the time of subsequent glaucoma surgery. Eight additional patients (2.0%) required MicroShunt repositioning at the time of open revision.

Discussion

The results of this 2-year prospective, randomized trial comparing the effectiveness and safety of the

MicroShunt versus trabeculectomy in eyes with medically uncontrolled POAG showed that IOP reduction and surgical success rates were statistically greater in the trabeculectomy group, similar to the results observed after 1 year.[13] The rates of surgical success, defined as a ≥20% reduction in baseline IOP without an increase in the number of glaucoma medications, at 2 years were 64.4% in the trabeculectomy group (72.7% at year 1) and 50.6% (53.9% at year 1) in the MicroShunt group. These rates are generally consistent with studies reporting the outcomes of these 2 procedures individually.[17, 18,19,20,21,22,23,24] Several retrospective, multicenter studies have reported that the 1-year overall success rates of the MicroShunt ranged from 59% to 74%, depending on the definition of success.[17,18,19] A prospective multicenter study reported that the 1-year overall success rate of the MicroShunt was 78%,[20] whereas retrospective single-center studies have reported overall success rates (with or without medication) of 79% at both 1 year and 2 years.[21] The success rates of trabeculectomy at 1 and 3 years were 87% and 79%, the Tube Versus Trabeculectomy respectively, in trial, [22,23] and 92% and 67%, respectively, in the Primary Tube Versus Trabeculectomy trial.[4,24] A post hoc analysis of the data from the present study stratified patients into tertiles based on baseline IOP. The greatest difference in surgical success between the MicroShunt and trabeculectomy groups was found in patients with a baseline pressure between 18 and 21 mmHg (Fig 2). Patients with a low baseline IOP (< 18 mmHg) were least likely to achieve surgical success in either group, with only 36.1% of patients in the MicroShunt group and 48.9% of patients in the trabeculectomy group meeting the primary end point. Inability to safely titrate the IOP to a low number with either procedure may have contributed to the relatively high rate of failure with both procedures in this subset of patients.

A variety of factors account for differences in surgical success when comparing studies, including but not limited to the definition of success, patient demographics and characteristics, surgeon experience and skill, and fixed parameters of the procedure itself. When performing microshunting procedures, pharmacologic inhibition of post- operative fibrosis is critical to achieving and maintaining optimal IOP lowering,[8] because the device dimensions limit flow and there are no other ways to manipulate bleb formation. Antifibrosis therapy with MMC or 5-fluorouracil is often used both intraoperatively and postopera tively to minimize scarring. The specific antimetabolite used, as well as its dose and method of delivery, is standardized within studies but differs between studies.[17, 18, 19, 20, 21, 22] In the current pivotal trial, 0.2 mg/ml of MMC was applied to the scleral bed for 2 minutes, according to a protocol finalized in 2017. Subsequently, the results of Schlenker et al[25] have suggested that for MicroShunt implantation, an MMC concentration of 0.2 mg/ml is associated with a higher risk of surgical failure compared with a concentration of 0.4 to 0.5 mg/ml (hazard ratio, 2.51; 95% CI, 1.01–6.23). Likewise, a 2-year, nonrandomized, multicenter study of the MicroShunt device showed that 90.3% of patients treated with MMC 0.4 mg/ml were medication-free, compared with 51.9% of patients treated with MMC 0.2 mg/ml.[20] It is possible that outcomes in the MicroShunt arm of the current study would have been improved had a higher dose of MMC been used, especially in patients at higher risk of failure due to scarring. It is interesting that the rate of surgical success with the MicroShunt was substantially different between the United States (47.5%) and Europe (69%), whereas the surgical technique and MMC concentration and delivery were the same. The significantly higher percentage of Black patients in the US cohort may have contributed to this difference, because Black race is known to be a risk factor for worse outcomes after glaucoma surgery.[26,27]

The most common reason for surgical failure through 2 years of follow-up in trabeculectomy-treated eyes was persistent hypotony (15.2% vs. 3.8% in MicroShunt-treated eyes), defined as IOP < 6 mmHg at 2 consecutive visits after 3 months. In contrast, the most common cause of surgical failure in MicroShunt-treated eyes was an IOP reduction of less than 20% from baseline (29.4% vs. 9.1% in trabeculectomy-treated eyes). These data provide evidence of the relative protection against hypotony afforded by the inherent resistance to fluid flow of the MicroShunt device. Conversely, limitations imposed by this inherent resistance may predispose to episcleral fibrosis and IOP elevation, particularly in patients at higher risk for postoperative scarring. Postoperative needling at the slit lamp was performed in 98 patients (24.8%) in the MicroShunt group and 12 patients (9.1%) in the trabeculectomy group (P < 0.001). When performing needle revision, surgeons were allowed to apply additional MMC at their desired dose. Of patients who underwent needling without prior glaucoma reoperation, 25.3% (24/95) of patients in the MicroShunt arm and 54.5% (6/11) of patients in the

trabeculectomy arm went on to achieve surgical success. Likewise, open bleb revision in the operating room allowed for surgeons to place additional MMC at the time of the procedure. Forty patients (10.1%) underwent open revision in the operating room in the MicroShunt group, and 10 patients (7.6%) required revision in the trabeculectomy arm. Among patients who underwent bleb revision without prior reoperation, 33.3% (13/39) in the MicroShunt group and 0% (0/10) in the trabeculectomy group (P = 0.045) went on to need traditional glaucoma surgery, including placement of a glaucoma drainage device or trabeculectomy by the end of year 2.

When selecting therapy, the risks of each treatment should be weighed against patient-specific treatment goals. A procedure that carries a higher risk of serious complications such as hypotony may be preferred for patients with more advanced, sight-threatening glaucoma or for those at higher risk of surgical failure due to postoperative scarring if this procedure is more likely to result in lower interventional IOP. Conversely, a safer procedure may be better for patients with more modest pressure goals and those at lower risk for filtration failure. The nature and rates of adverse events at 2 years were similar in the 2 groups, except for hypotony, which was more common in eyes undergoing trabeculectomy compared with MicroShunt implantation (51.1% vs. 30.9%, P < 0.001). Adverse events, such as a shallow anterior chamber and choroidal effusions, were transient and generally resolved with conservative management. Only 1 eye in the trabeculectomy group required drainage of choroidal effusions. At year 2, cataract progression was observed in 14.2% of eyes in the MicroShunt group and 21.4% of eyes in the trabeculectomy group (P = 0.071). No serious bleb-related complications, such as blebitis or bleb-related endophthalmitis, were observed in either group.

The skill and experience of the surgeon have a significant effect on the outcome obtained with any surgical procedure. This is particularly true for a procedure such as trabeculectomy that requires a delicate series of operative and postoperative manipulations and adjustments to achieve a positive result. Although trabeculectomy is still regarded as standard first-line treatment for patients requiring glaucoma surgery, the number of trabeculectomies performed each year continues to decrease while the number of microinvasive glaucoma surgeries increases.[28] Risks of the procedure as well as intraoperative and postoperative challenges are typically cited as the main reasons surgeons are moving away from trabeculectomy. The trabeculectomy arm of the present study demonstrated exceptional outcomes with respect to IOP reduction: The mean IOP at 2 years was 10.7 mmHg, using an average of 0.4 medications. Additionally, few serious complications were observed. All surgeons who participated in this trial had extensive experience with trabeculectomy. The benefits of MicroShunt procedures may include a reduction in the number, variability, and complexity of surgical manipulations, as well as a reduced need for postoperative adjustments. It is possible that surgeons with less experience performing and managing trabeculectomy would experience outcomes that are not as favorable as those obtained in this study and closer to those achieved with the MicroShunt. The relative performance of MicroShunt might be better in surgeons with limited trabeculectomy experience, but this needs to be confirmed with future studies.

Study Limitations

This study had several limitations. Although subjects were masked to treatment assignment, masking of surgeons was not feasible. Baseline IOP was < 18 mmHg in 31% of eyes (no washout before surgery), which may have led to the relatively high rates of failure in both groups. Therefore, the chosen primary outcome measure may not have been an ideal measure for determining surgical success. This is supported by the finding that the percentage of patients in each group who were medication-free was higher than the percentage who achieved overall surgical success. An additional study limitation may have been the use of MMC-soaked sponges at a fixed concentration for a fixed period of time. Future studies comparing the MicroShunt and trabeculectomy that use varying concentrations of MMC and different delivery methods (e.g., via injection) may yield different results.

Conclusions

This prospective, randomized, single-masked trial showed that both trabeculectomy and MicroShunt implantation resulted in significant and sustained IOP reduction at year 2, with trabeculectomy continuing to result in greater surgical success based on the primary end point.

Acknowledgments

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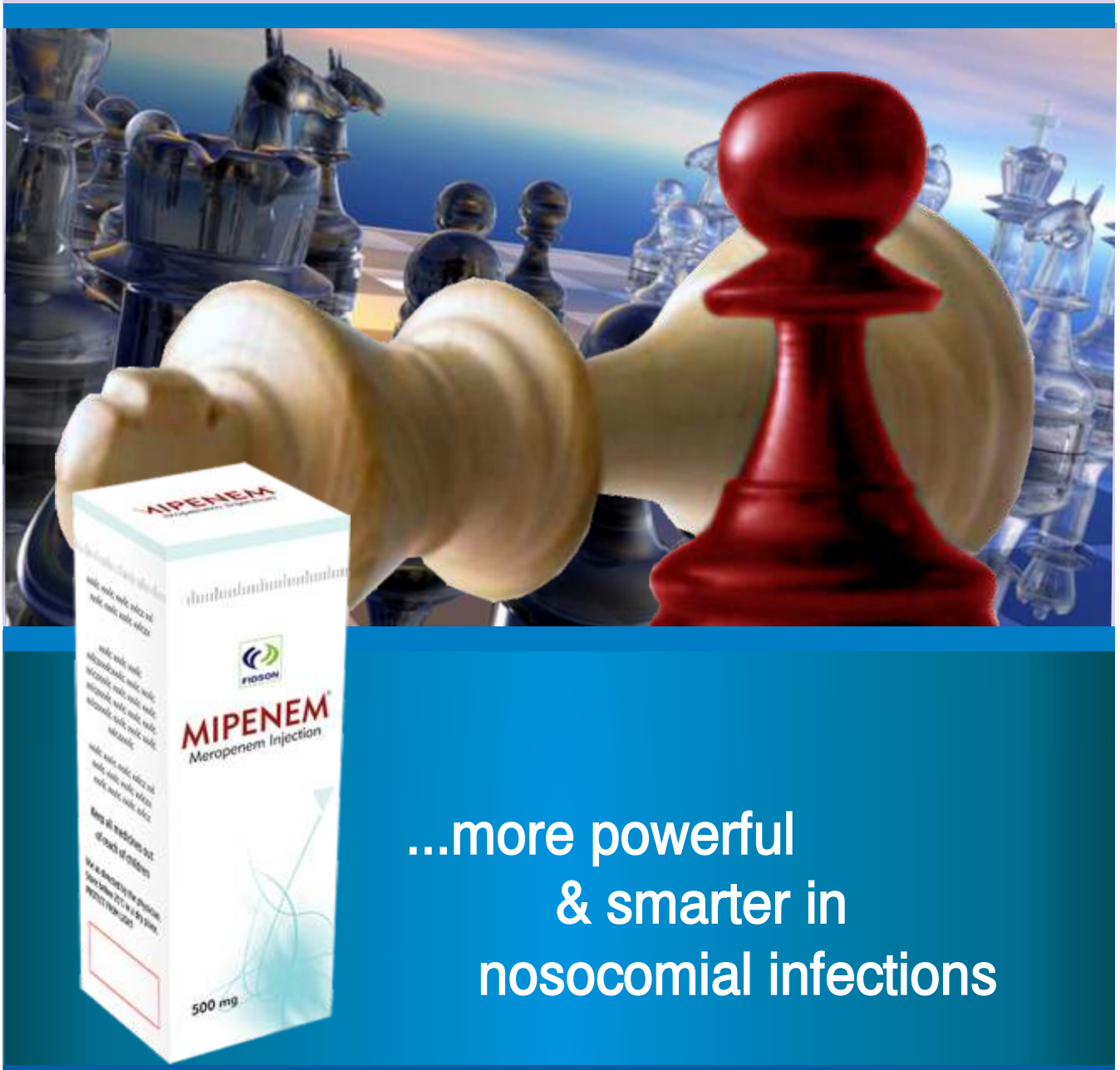

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