Abstract

Objectives: Phase angle (PA) constitutes a bioelectrical impedance measurement, indicating cell membrane health and integrity, hydration, and nutritional status. Handgrip strength (HS) has been also associated with body composition, nutritional status, inflammation, and functional ability in several chronic diseases. Although their prognostic significance as independent biomarkers has been already investigated regarding the outcomes of a cardiac surgery, our study is the first one to assess the combined predictive value of preoperative PA and HS.

Design and methods: HS and PA measurements were performed preoperatively in 195 patients undergoing cardiac surgery. The association of the combination of HS and PA with all-cause mortality rates was the primary study outcome, while its association with the intensive care unit (ICU) length of stay (LOS) was the secondary one.

Results: PA was positively correlated with HS ($r = 0.446$, $p < 0.005$) and negatively with EuroSCORE II ($r = -0.306$, $p < 0.005$). The combination of PA < 5.15 and HS < 25.5 was associated with higher one-year all-cause mortality (OR = 9.28; 95% CI 2.50–34.45; $p = 0.001$) compared to patients with PA > 5.15 and HS > 25.5, respectively. Patients with combined lower values of PA and HS (PA < 5.15 and HS < 30.7) were at higher risk of prolonged ICU LOS (OR = 4.02; 95% CI 1.53–10.56; $p = 0.005$) compared to those with higher PA–HS (PA > 5.15–HS > 30.7). The combination of PA–HS was also significantly linked with EuroSCORE II.

Conclusion: The combination of low preoperative PA and HS values was significantly associated with higher risk of all-cause mortality at 12 months and prolonged ICU LOS; thereby it might serve as a clinically useful prognostic biomarker after cardiac surgery procedures.

Keywords: Cardiac surgery, Phase angle, Handgrip strength, Coronary artery bypass grafting, All-cause mortality

Introduction

Predicting post-procedural morbidity and mortality remains challenging despite the development of many scoring systems and prognostic algorithms in cardiac surgery [1, 2]. Hence, the assessment of novel biomarkers capable of guiding the modern physician in choosing the optimal, individualized, treatment for patients based on clinical prediction is of utmost importance [3].

The latest international guidelines support a more individualized patient management based on group discussion among cardiac specialists (i.e. “heart team”) and on the utilization of novel biomarkers such as frailty tests [4]. Indicators of cellular integrity, functional capacity, and biological vulnerability have been recently proposed as preoperative risk factors associated with short- and long-term patient outcomes [5]. In this context, phase...
Materials and methods
Study population and eligibility criteria
The study population comprised adult patients undergoing scheduled selective coronary artery bypass grafting (CABG) surgery, individual valve replacement or repair, or any combination of these procedures at the Cardio-Thoracic Surgery Department of AHEPA University Hospital between December 2018 and October 2019.

The exclusion criteria were: i. age < 18 years, ii. hemodynamic instability requiring urgent surgery, iii. urgent surgery for aortic dissection, iv. any major adverse intra-operative outcome, v. congenital heart disease, vi. recent cardiac surgery during the prior three months, and vii. presence of any implantable device. The study protocol has been approved by the Scientific Board of AHEPA University Hospital as well as by Ethics Committee of the Aristotle University of Thessaloniki. Written informed consent was obtained pre-operatively from every participant.

Data extraction
On admission, demographic, anthropometric and clinical data [age, gender, and body mass index (BMI), type of surgery, EuroSCORE II, left ventricular ejection fraction (LVEF) and comorbidities] were recorded for each individual. Hospitalization data such as cardiopulmonary bypass (CPB) time, duration of mechanical ventilation, occurrence of post-operative complications, length of stay in the Intensive Care Unit (ICU) and postoperative length of hospital stay were also noted.

PA was measured using bioelectrical impedance method on the first pre-operative day by a blinded researcher trained in this technique. A simple quadrupole measurement was performed to the right side of the body using four-surface electrodes (QuadScan 4000, Bodystat, Isle of White, UK). Thereby, resistance (restriction of current flow) and reactance (capacitance of cell membranes to block current) were measured. The primary 50 kHz resistance and reactance data were used to calculate PA (tangent of reactance / resistance X 180°, divided by p and expressed in degrees).

HS was also assessed preoperatively using a portable hydraulic dynamometer (Takei 5001 GripA, Takei Scientific Instruments CO, Japan). The HS test was performed in the sitting position, having their elbow flexed at 90°, whilst pressing the dynamometer with the dominant hand at full force for three seconds. After three repetitions of the test with an interval of one minute to avoid fatigue, the best performance was recorded in kilograms (kg).

Outcomes of interest
Primary study outcome was deemed all-cause mortality, as assessed at last available telephonic follow-up or as determined by electronic medical health records. The length of ICU stay was considered as the secondary study outcome.

Statistical analysis
Continuous variables are presented with mean and standard deviation (SD) or with median and intra-quartile range (IQR) depending on data normality. Categorical variables are presented with frequencies (n) and percentages (%). Regularity of data distribution was checked using the Kolmogorov–Smirnov test.

The correlation of PA and HS with outcomes of interest was performed via t-test or Mann Whitney test for independent samples. Logistic regression was performed to detect the independent effect of demographic and clinical indicators on the outcomes of interest. The predictive value of PA and HS was evaluated through receiver operator characteristics (ROC) analyses and calculated Areas Under the Curve (AUC). Cut-off points of PA and HS that maximize sensitivity and specificity for risk stratification were evaluated via calculating Positive Predictive Value (PPV) and Negative Predictive Value (NPV). Due to the non-standardization of PA and HS values, statistical tests of collinearity were executed for each one of the performed multivariate analyses to investigate if there is any source of collinearity which could decrease the value of the independent effect. All statistical analyses were performed with the statistical package SPSS version 21 (IBM Corporation, Somers, NY, USA). The p-value of less than 0.05 was defined as the level of statistical significance.
Results
Our study included 195 patients with a mean age of 67 years (SD: 9 years) of whom 150 were men (76.9%) and 45 women (23.1%). Of included patients, 90 suffered from coronary artery disease (46.2%), 87 (44.6%) from valvular disease and 18 from both (9.2%). Median EuroSCORE II was equal to 2.6%(0.6–15.5). Demographic and clinical characteristics of the overall study population and of the patients who survived compared to those who died during follow-up, are presented in Tables 1 and 2, respectively. PA was positively correlated with HS ($r = 0.446$ $p < 0.005$) and negatively with EUROSCORE

| Table 1 | Demographic and clinical characteristics of the overall study population |
|---------|-------------------------------------------------------------------------|
| Age (years) | 67.18 ± 9.14 |
| Male gender (%) | 150(76.9) |
| Body Mass Index (kg/m²) | 27.75 ± 4.34(18.1–39.6) |
| Cardiopulmonary bypass time (minutes) | 114.5(23–290) |
| Coronary Artery Disease/Valvular disease / both (%) | 90(46.2)/87(44.6)/18(9.2) |
| Type II diabetes mellitus (%) | 89(45.6) |
| Chronic kidney disease (%) | 66(33.8) |
| Euroscore II (units) | 2.6 (0.6–15.5) |
| Phase angle (°) | 5.52 ± 1.47(2.7–160) |
| Handgrip strength (calf circumference) | 27.48 ± 9.16(5–51) |
| Postoperative infections (%) | 22(11.3) |
| Intensive Care Unit stay over 1 day (%) | 99(50.8) |
| Mechanical ventilation over 1 day (%) | 51(26.2) |
| In-hospital postoperative stay more than 7 days (%) | 147(75.4) |
| Postoperative complications (reopening, pulmonary embolism, peripheral thrombosis, septic condition, in-hospital mortality) (%) | 51(26.2) |
| All-cause mortality (%) | 27(13.8) |

Continuous variables are recorded as mean ± standard deviation or median (interquartile range), while categorical ones as n (%)

| Table 2 | Comparison of demographic and clinical characteristics between patients who survived and patients who died during follow-up |
|---------|----------------------------------------------------------------------------------------------------------------|
| | Dead patients (n = 27) | Alive patients (n = 168) | p value (dead vs alive) |
| Age (years) | 69.2 ± 7.6 | 66.9 ± 9.3 | 0.08 |
| Male gender | 23 (85%) | 127 (76%) | 0.37 |
| Body Mass Index (kg/m²) | 27.8 ± 4.8 | 27.8 ± 4.4 | 0.82 |
| Cardiopulmonary bypass time (minutes) | 135 ± 53.8 | 118 ± 44.2 | 0.17 |
| Type of surgery: | | | 0.45 |
| Coronary Artery disease | 11 (41%) | 79 (47%) | |
| Valvular disease | 12 (44%) | 75 (45%) | |
| Combined | 4 (15%) | 14 (8%) | |
| Type II diabetes mellitus | 12 (44%) | 77 (46%) | 0.84 |
| Chronic kidney disease | 9 (33%) | 57 (34%) | 0.48 |
| Euroscore II (units) | 3.8 (2.1) | 2.4 (1.8) | < 0.01 |
| Phase angle (units) | 5 ± 1.4 | 5.6 ± 1.5 | 0.04 |
| Handgrip strength (units) | 22.6 ± 8.4 | 28.1 ± 9.1 | < 0.01 |
| Post-operative infections | 12 (44%) | 10 (6%) | < 0.01 |
| Intensive Care Unit stay over 1 day | 22 (81%) | 77 (46%) | < 0.01 |
| Mechanical ventilation over 1 day | 20 (74%) | 31 (18%) | < 0.01 |
| In-hospital post-operative stay more than 7 days | 21 (78%) | 126 (75%) | 0.20 |

Continuous variables are recorded as mean ± SD or median (IQR)
II ($r = -0.306 \ p < 0.005$) (Table 3). All patients were followed up for a median period of 1 year and no patients were lost during follow-up (drop-out rate = 0%). The median hospital and ICU stay were 9 days (IQR: 3 days) and 2 days (IQR: 2 days), respectively.

Regarding the primary study outcome, 27 patients (13.8%) died from any cause after one-year follow-up. PA, HS and their combination had a significant yet fair predictive value for all-cause mortality; PA: AUC (95% CI) 0.657 (0.54–0.77); $p = 0.009$, HS: AUC (95% CI) 0.659 (0.5–0.78); $p = 0.008$, and their combination: AUC (95% CI) 0.671 (0.56–0.78); $p = 0.004$ (Table 4).

The PA–HS combination had a significant effect on mortality occurrence [$p = 0.009$]. Patients with PA < 5.15 and HS < 25.5 were 5 times more likely to die [5.13 (1.84–14.27); $p = 0.002$] when compared to those with PA > 5.15–HS > 25.5 (Table 5). The multivariate analysis, presented in Table 6, yielded that female gender [0.27 (0.08–0.94); $p = 0.040$], Euroscore II [1.59 (1.17–2.14); $p = 0.003$] and the combination of PA–HS [$p = 0.005$] had an independent effect on mortality. Regarding the PA–HS combination, patients with PA < 5.15 and HS < 25.5 were 9.3 times more likely to die [9.28 (2.50–34.45); $p = 0.001$] in relation to those with PA > 5.15–HS > 25.5 (Table 6).

A multivariate regression model for the prediction of 1-year mortality using PA and HS as continuous variables was also performed and is presented in Additional file 1: Table S1. According to this analysis, increased HS was independently linked with decreased all-cause mortality rates (aOR = 0.90, 95% CI: 0.84–0.96).

With regard to the secondary study outcome, the PA–HS combination was significantly associated with prolonged stay in the ICU [$p = 0.002$]. Patients with PA < 5.15 and HS < 30.7 were 4 times more likely to stay in the ICU for more than 1 day [4.14 (1.95–8.80); $p = 0.001$] in comparison with those having PA > 5.15 and HS > 30.7 (Table 7). PA, HS and their combination had also a significant yet poor predictive value for the prolonged stay in the ICU; PA: AUC (95% CI) = 0.600 (0.52–0.68); $p = 0.016$, HS: AUC (95% CI) = 0.586

### Table 3 Correlation of the PA indicator with HS and EuroScore II

| Correlation Indicators | PA Correlation Coefficient | $p$ value | N |
|------------------------|-----------------------------|-----------|---|
| Spearman’s rho HS      | 0.446                       | <0.005    | 195|
| EUROSCORE II(%)        | -0.306                      | <0.005    | 195|

**Bold values represent statistically significant values ($p < 0.05$)**

### Table 4 ROC analysis of the PA and HS indicators and their combination in relation to mortality and ICU stay

| Predictors | Variable     | AUC (95% CI) | $p$ value | Cut-off point | Sensitivity | Specificity | PPV* | NPV* |
|------------|--------------|--------------|-----------|---------------|-------------|-------------|------|------|
| PA†        | Mortality    | 0.657 (0.540.77) | 0.0090.016 | 5.15          | 67%         | 61%         | 21%  | 92%  |
|            | ICU LOS      | 0.600 (0.520.68) |           | 5.15          | 56%         | 70%         | 65%  | 60%  |
| HS†        | Mortality    | 0.659 (0.550.78) | 0.008     | 25.5          | 67%         | 63%         | 22%  | 92%  |
|            | ICU LOS      | 0.586 (0.510.67) | 0.040     | 30.75         | 71%         | 47%         | 57%  | 60%  |
|            | ICU LOS      | 0.586 (0.510.67) | 0.040     | 30.75         | 71%         | 47%         | 57%  | 60%  |
| Combination| PA/HS        | 0.671 (0.560.78) | 0.004     | 5.15/25.5     | 67%         | 62%         | 22%  | 92%  |
|            | ICU LOS      | 0.597 (0.520.68) | 0.019     | 5.15/30.75    | 63%         | 59%         | 61%  | 60%  |

**Bold values represent statistically significant values ($p < 0.05$)

* Lower values indicate a poor outcome

† AUC: Area Under the Curve; CI: confidence interval; PPV: positive prognostic value; NPV: negative prognostic value

### Table 5 Univariate logistic regression of all-cause mortality with the combination of PA and HS

| Predictors | OR 95% CI | $p$ value |
|------------|-----------|-----------|
| Combination| PA–HS     | 0.009     |
| PA > 5.15–HS > 25.5 | 1.00 (reference) | – |
| PA > 5.15–HS < 25.5 | 0.38 | 6.99 | 0.514 |
| PA < 5.15–HS > 25.5 | 0.33 | 5.95 | 0.655 |
| PA < 5.15–HS < 25.5 | 1.84 | 14.27 | 0.002 |

### Table 6 Multivariate logarithmic regression of all-cause mortality with the combination of PA and HS adjusted to clinical indicators

| Variables | OR 95% CI | $p$ value |
|-----------|-----------|-----------|
| Female Gender | 0.27 | 0.08 | 0.94 | 0.040 |
| Age       | 1.00     | 0.94     | 1.05 | 0.869 |
| Diabetes II | 0.85 | 0.31 | 2.30 | 0.746 |
| Chronic Kidney Disease | 0.53 | 0.19 | 1.50 | 0.233 |
| Euroscore II | 1.59 | 1.17 | 2.14 | 0.003 |
| Ejection Fraction | 0.96 | 0.91 | 1.02 | 0.226 |
| Combination PA–HS | 0.005 | – |
| PA > 5.15–HS > 25.5 | 1.00 (reference) | – |
| PA > 5.15–HS < 25.5 | 0.57 | 15.41 | 0.195 |
| PA < 5.15–HS > 25.5 | 0.34 | 8.02 | 0.533 |
| PA < 5.15–HS < 25.5 | 2.50 | 34.45 | 0.001 |

With regard to the secondary study outcome, the PA–HS combination was significantly associated with prolonged stay in the ICU [$p = 0.002$]. Patients with PA < 5.15 and HS < 30.7 were 4 times more likely to stay in the ICU for more than 1 day [4.14 (1.95–8.80); $p = 0.001$] in comparison with those having PA > 5.15 and HS > 30.7 (Table 7). PA, HS and their combination had also a significant yet poor predictive value for the prolonged stay in the ICU; PA: AUC (95% CI) = 0.600 (0.52–0.68); $p = 0.016$, HS: AUC (95% CI) = 0.586

**Bold values represent statistically significant values ($p < 0.05$)**

† Lower values indicate a poor outcome

† AUC: Area Under the Curve; CI: confidence interval; PPV: positive prognostic value; NPV: negative prognostic value
(0.51–0.67); \( p = 0.040 \), and their combination: AUC (95% CI) = 0.597 (0.52–0.68); \( p = 0.019 \) (Table 4).

Multivariate analysis for the prediction of prolonged ICU stay demonstrated that female gender [0.35 (0.15–0.83); \( p = 0.017 \)], EuroSCORE II [1.71 (1.27–2.30); \( p < 0.005 \)], left ventricular ejection fraction [1.05 (1.01–1.10); \( p = 0.018 \)] and the combination of PA–HS [\( p = 0.038 \)] were independent predictors. Regarding the combination of those indicators, patients with PA < 5.15 and HS < 30.7 were found to be 4 times more likely to stay in the ICU for more than 1 day [4.02 (1.53–10.56); \( p = 0.005 \)] compared to those with PA > 5.15–HS > 30.7 (Table 8).

A multivariate regression model for the prediction of ICU stay using PA and HS as continuous variables was also performed and is presented in Additional file 1: Table S2. According to that analysis, increased HS was independently associated with decreased ICU LOS (aOR = 0.95, 95% CI: 0.90–0.99).

In order to observe the relationship between the PA–HS combination and the standard EuroSCORE II risk index, an analysis of variance (ANOVA) was performed, showing a statistically significant difference in EuroSCORE II values among individuals with PA < 5.15–HS < 25.5 compared to those with PA > 5.15–HS > 25.5 in mortality (Table 9).

Statistical tests of collinearity were also performed for each one of the multivariate analyses to investigate if there is any source of collinearity which could decrease the value of the independent effect (Additional file 1: Table S3). No significant source of collinearity was identified since every calculated Tolerance was greater than 0.2 and the calculated variance inflation factors (VIFs) were relatively low.

**Discussion**

The results of this prospective observational study suggest the potential prognostic value of the combined pre-operative PA–HS measurement as a new biomarker for predicting one-year mortality in cardiac patients undergoing selective cardiac surgery. According to our analyses, patients having a combination of low PA and HS values were 5 times more likely to die and 4 times more likely to remain in the ICU for more than one postoperative day, thus increasing postoperative morbidity and the likelihood of complications. Furthermore, the EuroSCORE II index, an internationally established risk index for death after cardiac surgery, was associated with the combination of PA–HS. To our knowledge, this is the first study examining whether the combination of PA and HS values is associated with one-year mortality rates and early morbidity in patients undergoing cardiac surgery, and could thereby enhance their pre-operative risk stratification.

From the literature review, low PA is associated with nutritional risk, increased morbidity and mortality in immunocompromised patients or patients with chronic kidney disease [8]. It has been particularly associated with poor functional status and worse prognosis in cancer patients [8]. PA is often lower than normal in diseased individuals, since infection, systemic inflammation or specific parameters of a disease may cause cell destruction and consequent reduction in PA [8, 9]. PA is positively correlated with total body protein, muscle

| Table 7 | Univariate logistic regression of prolonged stay in the ICU with the combination of PA and HS |
|---------|-----------------------------------------------|
| OR      | 95% CI | p value |
| Combination PA–HS |  | 0.002 |
| PA > 5.15–HS > 30.7 | 1.00 (reference) | – |
| PA > 5.15–HS < 30.7 | 2.01 | 0.93 | 4.36 | 0.076 |
| PA < 5.15–HS > 30.7 | 3.97 | 1.27 | 12.43 | 0.018 |
| PA < 5.15–HS < 30.7 | 4.14 | 1.95 | 8.80 | <0.005 |

| Table 8 | Multivariate logistic regression of prolonged stay in the ICU with the combination of PA–HS adjusted to clinical indicators |
|---------|-----------------------------------------------|
| Variables | OR | 95% CI | p value |
| Female gender | 0.35 | 0.15 | 0.83 | 0.017 |
| Age | 1.03 | 0.99 | 1.07 | 0.152 |
| Diabetes II | 1.57 | 0.81 | 3.06 | 0.182 |
| Chronic Kidney Disease | 0.54 | 0.26 | 1.12 | 0.096 |
| Euroscore II | 1.71 | 1.27 | 2.30 | <0.005 |
| Ejection Fraction | 1.05 | 1.01 | 1.10 | 0.018 |
| Combination PA–HS | 0.038 |
| PA > 5.15–HS > 30.7 | 1.00 (reference) | – |
| PA > 5.15–HS < 30.7 | 2.19 | 0.90 | 5.32 | 0.084 |
| PA < 5.15–HS > 30.7 | 2.97 | 0.85 | 10.46 | 0.090 |
| PA < 5.15–HS < 30.7 | 4.02 | 1.53 | 10.56 | 0.005 |

| Table 9 | EUROSORE II relationship with PA–HS combination index |
|---------|-----------------------------------------------|
| Combination PA–HS | Combination PA–HS | Sig |
| below 5.15–below 25.5 | below 5.15–above 25.5 | 1.000 |
| above 5.15–below 25.5 | above 5.15–above 25.5 | 0.558 |
| above 5.15–above 25.5 | above 5.15–below 25.5 | <0.001 |
| below 5.15–above 25.5 | above 5.15–above 25.5 | 1.000 |
| above 5.15–below 5.5 | above 5.15–above 25.5 | 0.119 |
| above 5.15–below 25.5 | above 5.15–above 25.5 | 0.932 |

Dependent variable: EUROSORE II (%)
mass, offering a qualitative dynamic aspect of the body’s functional state [10]. Moreover, low PA values have been associated with weakness, vulnerability (frailty) and mortality, regardless of age and other comorbidities [11, 12].

According to the BICS (Bioimpedance in Cardiac Surgery) study enrolling 277 patients undergoing major cardiac surgery in Canada, PA can independently predict early and midterm mortality after major cardiac surgery [6]. A PA cutoff of < 4.5° had the highest predictive value for 1-year mortality, and every 1° decrease in PA conferred an almost threefold higher risk of mortality. Our analysis yielded a cut-off point of PA = 5.15° for the prediction of increased mortality and ICU stay. Additionally, PA has been suggested as a dynamic marker with the potential to respond to targeted interventions aiming to restore adequate nutritional status, increase physical activity, and optimize fluid status [6].

The measurement of HS is the most commonly used indicator for muscle function in several clinical conditions, as it is considered a strong indicator of functional capacity of the muscles as well as an indicative point of a patient’s nutritional status. The correlation between nutritional status and HS is well documented [13, 14]. Previous studies have also shown that HS was correlated with the severity of the disease, with aging and mortality in elderly individuals [15, 16]. Particularly in cardiac surgery, HS has been well-recognized as a preoperative risk stratification tool since weak HS has been associated with 1-year and 30-day mortality, heart failure, kidney disease, malnutrition, and various frailty scales [17–19]. Hence, our study concurs with the growing body of literature regarding the poor outcomes of cardiac surgery patients with low preoperative PA and HS, and adds that their combined assessment might be an option to consider as a risk stratification tool.

Nevertheless, our study is subject to several limitations. The small sample size, the limited follow-up and the monocentric study design restrict the generalizability of our findings. Secondly, we could not precisely record the dates of patients’ deaths and, therefore, survival analyses could not be performed. Furthermore, major adverse cardiovascular events and re-admissions were not documented in our database. Additionally, PA was not standardized for its direct determinants: age, gender and BMI, which could affect our results; however, we have adjusted our multivariate analyses for age and gender, while BMI was not univariately associated with either mortality or ICU LOS. Nevertheless, PA standardization prior to statistical analyses might be considered in future studies [20, 21]. Moreover, the characterization of prolonged ICU-stay as staying in ICU for more than 1 day was based on relevant studies [22–25], but not analyzing it as a continuous variable might cause potential misinterpretation of our results. However, PA and HS have not been sufficiently studied in the specific patient population; hence, our results seem to be promising for their utilization and could trigger future studies to combine these biomarkers and associate them with postoperative prognosis. Thereby, we could ultimately achieve a more detailed holistic risk stratification of patients undergoing cardiac surgery and possibly direct them towards other alternative treatments such as angioplasty, valvular replacement or optimal palliative care.

Conclusions
In this prospective observational study, the combination of low preoperative PA and HS values was significantly associated with higher risk of all-cause mortality at 12 months and prolonged ICU stay. Hence, these clinical biomarkers could serve as prognostic tools for assessing adverse clinical course after cardiac surgery procedures. Larger studies and randomized-controlled trials are needed to confirm these results.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s13019-022-01970-z.

Additional file 1: Supplementary tables S1, S2, and S3.

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Author contributions
MP, ASP and DVM developed the draft of the manuscript and wrote the main text of the manuscript; EV and MP mainly contributed to the statistical analyses; EK, KA and AG revised and edited the manuscript; GT developed the concept and supervised the study progress; all authors have read and approved the final manuscript.

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Availability of data and materials
The dataset supporting the conclusions of this article is available from Georgios Tagarakis (e-mail: gtagarakis@gmail.com) upon reasonable request and with permission of AHEPA University Hospital.

Declarations
Ethics approval and consent to participate
Study protocol has been approved by the Scientific Board of AHEPA University Hospital of Thessaloniki (reference number: 06/09/2018) and by the Ethics Committee of the Aristotle University of Thessaloniki (reference number: 21/11/2018). All methods were performed in accordance with the Declaration of Helsinki. All participants have provided written informed consent prior to study participation.

Consent for publication
All participants have provided written informed consent for study publication.

Competing interests:
The authors declare no competing interests.
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