Transfusion of red blood cells does not impact progression-free and overall survival after surgery for ovarian cancer

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BACKGROUND: Allogeneic red blood cells (RBCs) have the potential to impact the immunosurveillance of the recipient and may therefore increase the risk of recurrence after cancer surgery. In this article the relationship between perioperative RBC transfusion and the risk of recurrence after ovarian cancer surgery is examined.

STUDY DESIGN AND METHODS: This is a retrospective cohort analysis of a prospective database of patients who underwent surgery due to primary ovarian cancer between 2006 and 2014 and who had no residual disease after surgery. Patients who did and did not receive perioperative RBC transfusion were compared. The primary endpoint was progression-free survival (PFS). Propensity score matching (PSM) and Cox proportional hazards regression (CPH) was used to control for between-group differences of prognostic determinants.

RESULTS: A total of 529 patients with a median follow-up of 51.4 months (95% CI, 46.1-56.5) were eligible for analysis. Of those, 408 patients (77.1%) received allogeneic, leukoreduced RBCs with a median of 4 units (IQR, 2-6) per patient. There was a strong selection bias of prognostic determinants between patients with and without transfusion. In unadjusted analysis, transfusion of RBCs was associated with an increased risk of cancer recurrence (hazard ratio [HR] of PFS 2.71 [95% CI, 1.94-3.77], $p < 0.001$). After bias reduction, transfusion of RBCs was no longer associated with an increased risk of cancer recurrence, neither in PSM-adjusted (HR 1.03 [95% CI, 0.59-1.80], $p = 0.91$), nor in multivariable CPH-adjusted analysis (HR 1.26 [95% CI, 0.85-1.86], $p = 0.23$).

CONCLUSION: Perioperative transfusion of RBCs did not increase the risk of recurrence after ovarian cancer surgery.

Despite commendable efforts to reduce perioperative blood transfusions over the last years, transfusion of allogeneic red blood cells (RBCs) is still an essential component of the treatment of the surgical patient.[1] Nonetheless, preclinical evidence suggests that the transfusion of RBCs may have deleterious immunomodulatory effects, termed transfusion-related immunomodulation (TRIM).[2] Although clinical evidence of TRIM effects in patients receiving RBCs is still inconclusive, considerable evidence exists that RBC products are capable to modulate immune cell function through a variety of mechanisms and mediators that may induce both, proinflammatory and immunosuppressive effects.[3] Immunosuppressive effects after transfusion of RBCs such as an impaired natural killer (NK)-cell[4] and T-cell function[5] may

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be particularly relevant for patients undergoing major surgery for cancer. In these patients, an intact immunosurveillance might be able to eliminate circulating tumor cells during surgery, minimize residual disease, and prevent early formation of micrometastases after complete tumor resection. In this respect, a perioperative transient attenuation of the NK-cell and cytotoxic T-cell mediated immunosurveillance has been associated with a higher rate of cancer recurrence in both animal models and clinical studies.[6–9] Thus, given the potential of allogeneic RBCs to impact the immunosurveillance of the patient, perioperative transfusion of RBCs may increase the risk of recurrence after curative cancer surgery.

Whereas former randomized controlled trials—comparing allogeneic versus autologous and non-leukoreduced versus leukoreduced RBCs, respectively—found no evidence of RBC transfusion to increase the risk of cancer recurrence,[10–12] previous meta-analyses of retrospective observational studies comparing surgical oncology patients receiving RBCs with patients not receiving RBCs indicated a higher risk for cancer recurrence among transfused patients.[13–17] However, these results have to be interpreted carefully because many observational studies did not include an appropriate control for between-group differences of prognostic determinants. Therefore, it remains questionable whether perioperative RBC transfusions increase the risk of recurrence after curative surgery.

We hypothesized that perioperative transfusion of RBCs worsens outcome after surgery for cancer. The objective of this study was to investigate the impact of perioperative RBC transfusion on cancer recurrence and overall survival in patients undergoing ovarian cancer surgery, one of the surgical patient populations with a very high risk for allogeneic RBC transfusions during hospitalization.

METHODS

Study design and setting

This retrospective cohort study included patients who underwent surgery due to primary epithelial ovarian cancer (EOC) from January 2006 through January 2014 at the Department of Gynecology, European Competence Center for Ovarian Cancer, Charité - Universitätsmedizin Berlin, Campus Virchow-Klinikum, Berlin, Germany. Patients were identified from the prospectively maintained Tumor Bank Ovarian Cancer database (TOC, www.toc-network.de), a database that acquires information on patients' demographics, tumor dissemination patterns, histology, treatments and outcome with a follow-up every 6 months after primary surgery. Patients who received a perioperative transfusion of RBCs were compared to patients who did not receive an RBC transfusion. A perioperative RBC transfusion was defined as a transfusion of at least one unit of packed RBCs in the period from the beginning of surgery to the discharge from hospital. During the study period, patients were only transfused with allogeneic, prestorage leukoreduced, and non-irradiated packed RBCs at a hemoglobin threshold of 9 g/dL. Ethical approval was obtained from the ethical committee of Charité - Universitätsmedizin Berlin (No. EA4/128/17).

Participants

Patients were eligible if they 1) underwent primary cytoreduction, completion operation, or interval debulking surgery due to primary EOC, fallopian tube carcinoma, or primary peritoneal carcinoma (all referred to as "EOC" in this study) between January 2006 and January 2014 at the Department of Gynecology, European Competence Center for Ovarian Cancer, Charité - Universitätsmedizin Berlin, Campus Virchow-Klinikum, Berlin, Germany, and 2) had complete cytoreduction after surgery in terms of no macroscopic tumor residuals. Patients were excluded if they 1) died during the perioperative course, or 2) had missing data in the most important prognostic determinants of ovarian cancer (Fig. 1).

Endpoints

The primary endpoint was progression-free survival (PFS). PFS was defined as the period of months from date of surgery to documentation of cancer recurrence or cancer-related death, whichever came first. Cancer recurrence was defined as appearance of any new lesions diagnosed in clinical examination, ultrasound, or CT scan. The secondary endpoint was overall survival (OS). OS was defined as the period of months between surgery and death resulting from any cause. Patients who were still alive or alive and without cancer recurrence were censored for OS and PFS, respectively, at the date of last follow-up.

Data collection and definitions

Patients' demographics, clinicopathological data, and treatments and outcome data were obtained from the TOC database and were complemented by anesthesiological, transfusion, and inpatient medical data using the anesthesiological database maintained by the Department of Anaesthesiology and Intensive Care Medicine, and the hospital data management system (SAP, Germany) of Charité - Universitätsmedizin Berlin. RBC storage data were obtained from the transfusion database maintained by the Institute of Transfusion Medicine, Charité - Universitätsmedizin Berlin.

Interval debulking surgery was defined as surgical cytoreduction after neoadjuvant chemotherapy, while completion operation was defined as subsequent operation after diagnostic laparoscopy, or suboptimal primary debulking without prior chemotherapy to complete surgical cytoreduction. The International Federation of Gynecology and

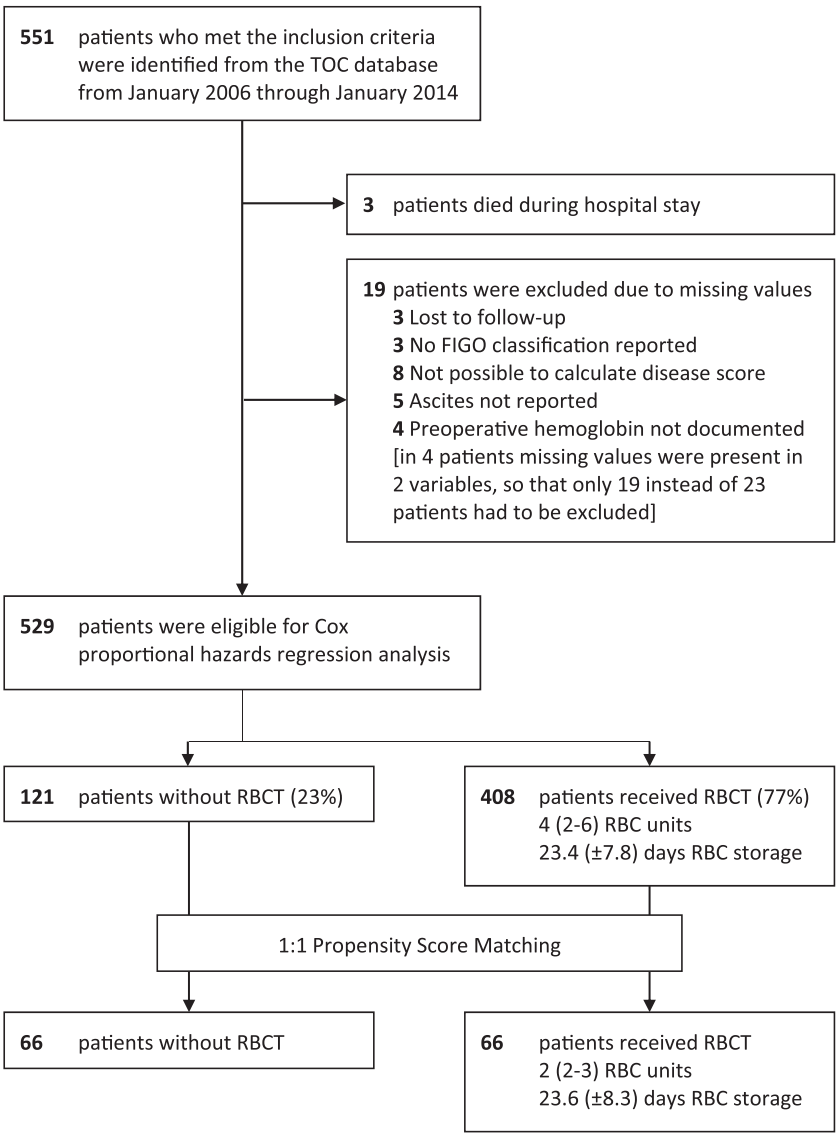

Fig. 1. Study flow diagram. RBCT, transfusion of RBCs.

Obstetrics (FIGO) classification used in this study was the one adopted before the modification applied in 2014.

Bias handling

Previous observational studies investigating the relation of perioperative transfusion of RBCs with oncological endpoints indicated a strong selection bias regarding baseline, clinicopathological and surgical characteristics when comparing patients with and without transfusion of RBCs.[18–20] Therefore, observational studies need to be conducted with high quality, ensuring that the most important prognostic determinants with regard to the specific study endpoints are captured in the available data and that appropriate analytical methods are performed to attenuate their confounding effects on specific endpoints. In this respect, we used a multivariable Cox proportional hazards regression (CPH) to control for between-group differences of prognostic determinants. Since goodness-of-fit measures do not allow to determine the degree to which the fitted regression model has successfully eliminated systematic differences between treated and untreated subjects, propensity score matching (PSM) was performed as complementary analysis.[21] In PSM, randomization is mimicked by optimally balancing confounders. The propensity score (PS) is the probability of treatment assignment conditional on the confounding variables. The PS allows analysis of an observational nonrandomized study in a way that it mimics some of the particular characteristics of a randomized controlled trial. Conditional on the PS, the distribution of the confounding variables will be similar between patients with and without transfusion of RBCs.

TABLE 1. Demographics and tumor data of the study population and the matched cohort

	All patients			Matched cohort		
Characteristic	No RBCT (n = 121)	RBCT (n = 408)	p value	No RBCT (n = 66)	RBCT (n = 66)	p value
Age (years)	52.0 (44.0-60.0)	58.0 (51.0-68.0)	<0.001	53.5 (46.2-64.8)	54.5 (47.0-64.5)	0.85
BMI (kg/m^2)	24.2 (21.3-27.9)	24.5 (21.8-28.1)	0.49	23.6 (21.2-26.6)	25.5 (21.3-29.7)	0.20
ASA stage, n (%)			0.002			0.25
ASA I	14 (12.0)	23 (5.8)		9 (14.1)	4 (6.2)	
ASA II	83 (70.9)	250 (63.0)		42 (65.6)	42 (65.6)	
ASA III	20 (17.1)	124 (31.2)		13 (20.3)	18 (28.1)	
Final Diagnosis, n (%)			0.32			0.60
Primary ovarian cancer	108 (89.3)	374 (91.7)		60 (90.9)	63 (95.5)	
Fallopian tube carcinoma	8 (6.6)	14 (3.4)		3 (4.5)	2 (3.0)	
Primary peritoneal carcinoma	5 (4.1)	20 (4.9)		3 (4.5)	1 (1.5)	
CA-125 (U/ml)	54 (22-266)	235 (53-1052)	<0.001	145 (25-397)	59 (20-231)	0.35
FIGO classification, n (%)			<0.001			0.86
Stage Ia	24 (19.8)	21 (5.1)		10 (15.2)	11 (16.7)	
Stage Ib	1 (0.8)	4 (1.0)		1 (1.5)	1 (1.5)	
Stage Ic	22 (18.2)	20 (4.9)		13 (19.7)	11 (16.7)	
Stage IIa	6 (5.0)	8 (2.0)		4 (6.1)	4 (6.1)	
Stage IIb	3 (2.5)	13 (3.2)		3 (4.5)	2 (3.0)	
Stage IIc	9 (7.4)	6 (1.5)		0 (0.0)	3 (4.5)	
Stage IIIa	10 (8.3)	10 (2.5)		5 (7.6)	4 (6.1)	
Stage IIIb	1 (0.8)	21 (5.1)		1 (1.5)	0 (0.0)	
Stage IIIc	39 (32.2)	255 (62.5)		25 (37.9)	28 (42.4)	
Stage IV	6 (5.0)	50 (12.3)		4 (6.1)	2 (3.0)	
Disease score, n (%)			<0.001			0.99
Low	82 (67.8)	94 (23.0)		36 (54.5)	36 (54.5)	
Moderate	23 (19.0)	92 (22.5)		16 (24.2)	16 (24.2)	
High	16 (13.2)	222 (54.4)		14 (21.2)	14 (21.2)	
Tumor dissemination pattern, n (%)						
Level 1, pelvic	84 (69.4)	356 (87.5)	<0.001	50 (75.8)	47 (71.2)	0.69
Level 2, extrapelvic	32 (26.4)	286 (70.3)	<0.001	26 (39.4)	26 (39.4)	0.99
Level 3, extrapelvic	19 (15.7)	231 (56.8)	<0.001	15 (22.7)	15 (22.7)	0.99
Malignant ascites, n (%)			<0.001			0.99
None	78 (64.5)	183 (44.9)		43 (65.2)	43 (65.2)	
<500 mL	38 (31.4)	126 (30.9)		18 (27.3)	19 (28.8)	
>500 mL	5 (4.1)	99 (24.3)		5 (7.6)	4 (6.1)	
Histological diagnosis, n (%)			<0.001			0.55
Serous papillary	92 (77.3)	346 (86.1)		52 (80.0)	51 (78.5)	
Mucinous	12 (10.1)	8 (2.0)		4 (6.2)	2 (3.1)	
Endometrioid	7 (5.9)	24 (6.0)		4 (6.2)	8 (12.3)	
Undifferentiated	0 (0.0)	5 (1.2)		0 (0.0)	0 (0.0)	
Clear cell	8 (6.7)	17 (4.2)		5 (7.7)	4 (6.2)	
Other	0 (0.0)	2 (0.4)		0 (0.0)	0 (0.0)	
Grade, n (%)			0.005			0.63
I	19 (16.0)	34 (8.5)		10 (15.4)	9 (14.1)	
II	38 (31.9)	95 (23.8)		19 (29.2)	24 (37.5)	
III	62 (52.1)	270 (67.7)		36 (55.4)	31 (48.4)	
HGSOC, n (%)	82 (67.8)	335 (82.1)	<0.001	46 (69.7)	47 (71.2)	0.99

Data are expressed as median (25%, 75% quartiles), or frequencies (%), as appropriate. p values were calculated using the Wilcoxon-Mann–-Whitney test and the Fisher's exact test, as appropriate.
BMI = body mass index; ASA = American Society of Anesthesiologists; FIGO = The International Federation of Gynecologists and Obstetricians; HGSOC = high grade serous ovarian cancer; RBCT = transfusion of RBCs.

Given the current evidence on prognostic determinants of cancer recurrence and survival in ovarian cancer patients, the following variables—with respect to the primary and secondary endpoints—were considered when controlling for between-group differences: 1) age, 2) FIGO stage, 3) type of surgery, 4) disease burden according to disease score,[22,23] 5) malignant ascites,[24] 6) high grade serous ovarian cancer (HGSOC), 7) surgical complexity score,[24,25] and 8) adjuvant chemotherapy. We further included 9) preoperative hemoglobin,[26] and 10) number of FFP units transfused during hospital stay,[27] because preoperative anemia and transfusion of fresh frozen plasma (FFP) might be further determinants involved in recurrent disease after surgery.

Statistical analyses

Statistical analyses were performed using the R project for Statistical Computing (Version 3.4.3, R-packages: Gmisc, Hmisc, tableone, MatchIt, rms, survival).

TABLE 2. Surgical data and adjuvant treatment of the study population and the matched cohort

	All patients			Matched cohort		
Characteristic	No RBCT (n = 121)	RBCT (n = 408)	p value	No RBCT (n = 66)	RBCT (n = 66)	p value
Debulking surgery, n (%)			<0.001			0.61
Primary debulking	57 (47.1)	253 (62.0)		36 (54.5)	31 (47.0)	
Completion debulking	55 (45.5)	87 (21.3)		23 (34.8)	25 (37.9)	
Interval debulking	9 (7.4)	68 (16.7)		7 (10.6)	10 (15.2)	
Surgical complexity, n (%)			<0.001			0.99
Low	29 (24.0)	30 (7.4)		14 (21.2)	14 (21.2)	
Intermediate	86 (71.1)	174 (42.6)		46 (69.7)	45 (68.2)	
High	6 (5.0)	204 (50.0)		6 (9.1)	7 (10.6)	
Duration of surgery (min)	200 (155-247)	265 (209-328)	<0.001	199 (159-250)	206 (165-248)	0.80
Preoperative hemoglobin (g/dl)	13.5 (12.7-14.1)	12.5 (11.6-13.4)	<0.001	13.1 (12.3-13.8)	13.1 (12.1-14.0)	0.96
RBC transfusion during hospital stay (units)	–	4.0 (2.0-6.0)	–	–	2.0 (2.0-3.0)	–
RBC storage (days)	–	23.4 (±7.8)		–	23.6 (±8.3)	–
FFP transfusion during hospital stay, n (%)	36 (29.8)	366 (89.7)	<0.001	33 (50.0)	32 (48.5)	0.99
FFP transfusion during hospital stay (units)	0.0 (0.0-2.0)	9.0 (4.0-15.0)	<0.001	1.0 (0.0-3.0)	0.0 (0.0-3.8)	0.82
Postsurgical ward, n (%)			<0.001			0.60
General ward	74 (63.2)	94 (24.2)		35 (53.0)	33 (51.6)	
Postanesthesia care unit	14 (12.0)	80 (20.6)		10 (15.2)	14 (21.9)	
Intensive care unit	29 (24.8)	214 (55.2)		21 (31.8)	17 (26.6)	
Hospital length of stay (days)	12.0 (10.5-14.0)	15.0 (13.0-21.0)	<0.001	13.0 (11.0-14.8)	14.0 (11.0-17.0)	0.13
Adjuvant chemotherapy, n (%)			<0.001			0.92
No chemotherapy	21 (17.4)	51 (12.5)		9 (13.6)	11 (16.7)	
Taxol/Carboplatin	81 (66.9)	335 (82.1)		47 (71.2)	45 (68.2)	
Other platinum-containing chemotherapy	17 (14.0)	20 (4.9)		8 (12.1)	9 (13.6)	
Other	2 (1.7)	2 (0.5)		2 (3.0)	1 (1.5)	
Platinum Sensitivity, n (%)			0.001			
Responsive	97 (80.2)	305 (74.8)		55 (83.3)	50 (75.8)	0.44
Non-responsive	3 (2.5)	52 (12.7)		2 (3.0)	5 (7.6)	
No chemotherapy	21 (17.4)	51 (12.5)		9 (13.6)	11 (16.7)	

Data are expressed as mean (standard deviation), median (25%, 75% quartiles), or frequencies (%), as appropriate. p values were calculated using the Wilcoxon-Mann–Whitney test and the Fisher's exact test, as appropriate.
Surgical complexity was assessed by Surgical complexity score.
FFP = fresh frozen plasma; RBCT = transfusion of RBCs.

To check the present data distributions, we used the graphic inspection by box plots, QQ plots, histograms, and arithmetically examined the distributions by skewness. Data were expressed as mean (standard deviation, SD), median (25%, 75% quartiles) or frequencies (%), as appropriate. Differences of continuous data were tested using one-way ANOVA or the exact Mann–Whitney U test for independent groups, and frequencies were tested using Fisher's exact test. To assess the risk of requiring perioperative transfusion of RBCs, multivariable logistic regression using a backward variable selection procedure based on the Akaike information criterion was performed.

To evaluate the relation of transfusion of RBCs with the primary and secondary endpoint, univariable CPH, multivariable CPH, and univariable CPH after PSM were performed. The multivariable CPH included the 10 prognostic determinants described in the bias handling section and used a backward variable selection procedure from the full regression model based on the Akaike information criterion. In case of violation of the proportional hazard assumption, the particular variable was entered as strata into the multivariable model. In PSM, PS was estimated by fitting a logistic-regression model that included the 10 prognostic determinants. Thereafter, a 1:1 pair matching between patients receiving and not receiving transfusion of RBCs was applied using the recommended method of nearest-neighbor matching without replacement,[28] with a caliper width equal to 0.1 of the standard deviation of the logit of the PS. The distribution of PS before and after matching was checked by histograms. The appropriateness of matching was assessed by comparing the standardized mean differences (SMD) of the prognostic determinants between patients receiving and not receiving transfusion of RBCs and by the percentage of balance improvement of the PS. A SMD of less than 0.1 was considered a negligible imbalance between the two groups. Kaplan–Meier methods were used to estimate and compare PFS and OS between patients with and without transfusion of RBCs before and

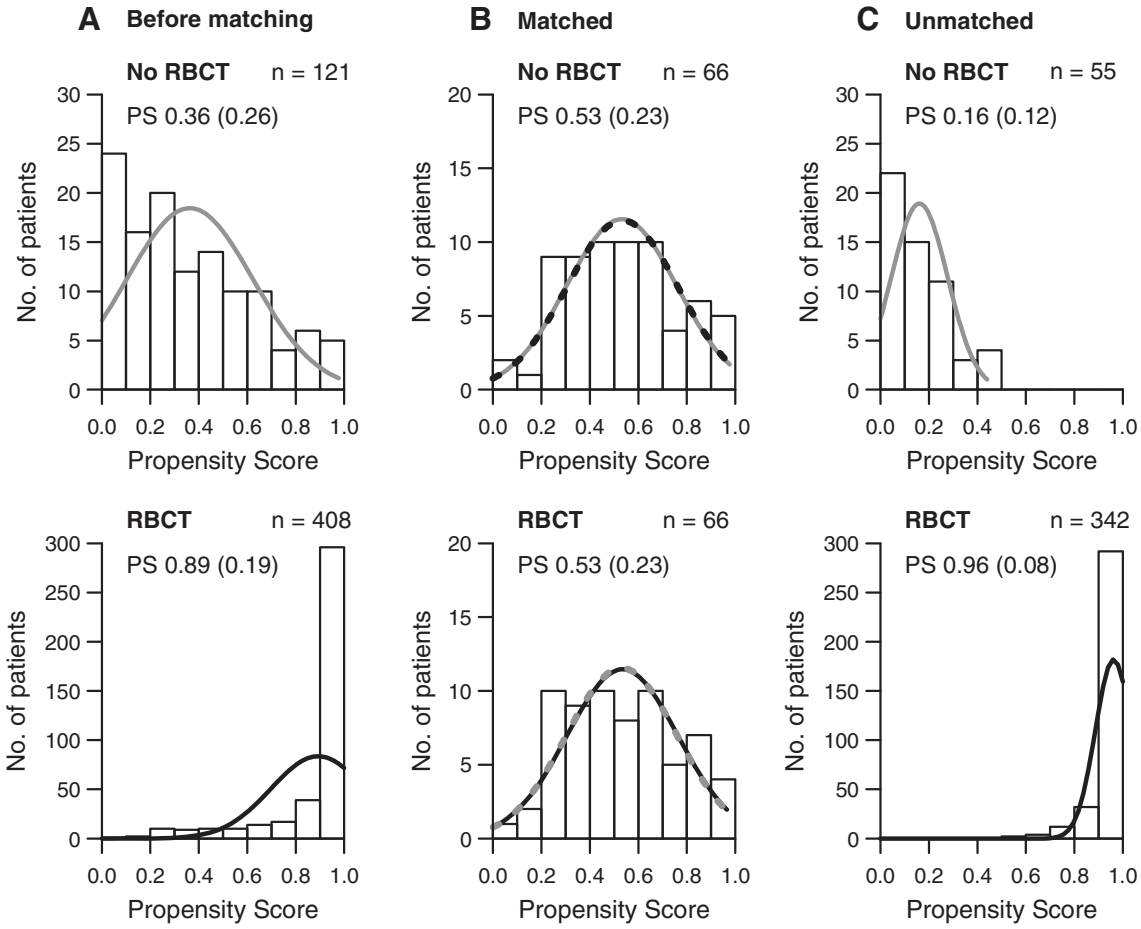

Fig. 2. Distribution of propensity scores (PS) in patients not receiving (upper figures) and patients receiving transfusion of RBCs (RBCT) (lower figures) in the entire cohort before matching (A), in the matched cohort (B), and in the cohort who could not be matched due to no matching partner available (C). The number of patients and the mean PS (with SD) in each cohort are indicated.

after PSM. The proportional hazard assumption was tested by scaled Schoenfeld residuals and by inspection of the hazard ratio (HR) plots. An equal distribution of censoring was checked and median follow up was calculated by inverse Kaplan Meier methods. A two-tailed p-value <0.05 was considered statistically significant.

RESULTS

A total of 551 patients met the inclusion criteria. Of those, three patients died during the hospital stay (0.5% [95% CI, 0.1-1.7]) and another 19 patients were excluded due to missing values of prognostic determinants that were required to control for between-group differences. Therefore, a total of 529 patients were eligible for analysis (Fig. 1).

Four hundred and eight patients (77.1% [95% CI, 73.2-80.6]) received a perioperative transfusion of RBCs including 286 patients (54.0% [95% CI, 49.7-58.4]) transfused during surgery, and 342 patients (64.7% [95% CI, 60.3-68.6]) transfused during the postoperative course. A total of 2111 units of RBCs were transfused, which equals a median of 4 units (25%; 75% quartiles, 2; 6) per patient. The mean RBC storage duration was 23.4 (±7.8) days. The median follow-up time of the study population was 51.4 months (95% CI, 46.1-56.5). A total of 137 patients (25.8% [95% CI, 22.2-29.8]) died during their follow-up, while 287 patients (54.2% [95% CI, 49.8-58.5]) had either cancer recurrence or cancer-related death. Five-year PFS and OS of the study population were 34.0% (95% CI, 29.5-39.2) and 66.8% (95% CI, 61.9-72.1), respectively. The median PFS was 30.6 months (95% CI, 26.1-36.4), whereas the median OS was not reached.

Risk for perioperative transfusion of RBCs

In the univariable analysis, most baseline, clinicopathological and surgical characteristics differed significantly between patients who received a perioperative transfusion of RBCs and patients who did not receive a perioperative RBC transfusion (Table 1 and Table 2, left columns). After

TABLE 3. Association of perioperative transfusion of RBCs and progression-free survival according to unadjusted, multivariable and propensity score matching adjusted analyses

Characteristic	Unadjusted*		Multivariable CPH adjusted†		PSM adjusted‡	
	HR (95% CI)	p value	HR (95% CI)	p value	HR (95% CI)	p value
Perioperative RBCT						
No	Reference	<0.001	Reference	0.23	Reference	0.91
Yes	2.71 (1.94-3.77)		1.26 (0.85-1.86)		1.03 (0.59-1.80)	
					Persisting bias after PSM SMD	p value
Age (per year)	1.01 (1.00-1.02)	0.015	1.00 (0.99-1.01)	0.33	0.099	0.85
FIGO classification						
Stage I	Reference		Reference		0.171	0.86
Stage II	2.73 (1.31-5.69)	0.007	2.14 (1.01-4.55)	0.04		
Stage III	6.46 (3.68-11.3)	<0.001	2.78 (1.42-5.42)	0.002		
Stage IV	8.48 (4.53-15.8)	<0.001	3.04 (1.42-6.36)	0.002		
Disease score						
Low	Reference		Reference		<0.001	0.99
Moderate	2.26 (1.54–3.30)	<0.001	1.12 (0.71-1.76)	0.62		
High	4.44 (3.22–6.12)	<0.001	1.95 (1.27-2.99)	0.002		
Malignant ascites						
None	Reference		Reference		0.065	0.99
<500 mL	1.35 (1.03-1.78)	0.028	1.28 (0.94-1.74)	0.10		
>500 mL	2.17 (1.63-2.90)	<0.001	1.44 (0.99-2.10)	0.05		
HGSOC						
No	Reference		Reference		0.039	0.99
Yes	2.28 (1.61-3.22)	<0.001	1.45 (1.00-2.09)	0.048		
Debulking surgery						
Primary debulking	Reference		Reference		0.173	0.61
Completion debulking	0.53 (0.39-0.72)	<0.001	1.11 (0.78-1.58)	0.55		
Interval debulking	1.87 (1.38-2.55)	<0.001	2.38 (1.62-3.50)	<0.001		
Surgical complexity score						
Low	Reference		not included		0.052	0.99
Intermediate	1.23 (0.77-1.96)	0.375	not included			
High	2.88 (1.82-4.55)	<0.001	not included			
Preoperative hemoglobin (per g/dl)	0.86 (0.80-0.93)	<0.001	0.95 (0.87-1.04)	0.32	0.052	0.96
FFP transfusion during hospital stay (per unit)	1.02 (1.01-1.02)	<0.001	1.00 (0.99-1.01)	0.15	0.056	0.82

* Univariable Cox proportional hazards regression analyses (patients n = 529).

† Multivariable Cox proportional hazards regression analyses using a backward variable selection procedure based on the Akaike information criterion (AIC) (patients n = 529, events n = 287). Adjuvant chemotherapy was included as strata due to violation of the proportional hazard assumption. According to AIC analyses, surgical complexity score was removed from the full model to provide the best model of fit.

‡ Univariable Cox proportional hazards regression analyses after propensity score matching (PSM) using all variables in calculation of the propensity score (patients n = 132). The persisting bias after PSM was evaluated by standardized mean differences (SMD).

FIGO = The International Federation of Gynecologists and Obstetricians; HGSOC = high grade serous ovarian cancer; FFP = fresh frozen plasma; RBCT = transfusion of RBCs.

multivariable adjustment, higher age (per year, odds ratio [OR] 1.06 [95% CI, 1.03-1.08], $p < 0.001$), longer duration of surgery (per hour, OR 1.51 [95% CI, 1.19-1.96], $p < 0.001$), lower preoperative hemoglobin levels (per g/dl, OR 1.95 [95% CI, 1.58-2.47], $p < 0.001$), higher disease score (moderate, OR 2.47 [95% CI, 1.31-4.80], $p < 0.001$; high, OR 5.21 [95% CI, 2.60-10.9], $p < 0.001$), and higher surgical complexity (intermediate, OR 1.41 [95% CI, 0.69-2.83], $p < 0.001$; high, OR 6.88 [95% CI, 2.27-23.1]) were independent predictors for receiving a perioperative transfusion of RBCs.

Perioperative transfusion of RBCs and endpoints

There was a strong selection bias regarding the prognostic determinants when patients transfused with RBCs were compared with patients without transfusion of RBCs (PS 0.89 ± 0.19 vs. 0.36 ± 0.26, $p < 0.001$, SMD 2.28, $p < 0.001$) (Fig. 2).

In unadjusted analysis, transfusion of RBCs was related to a 2.7-fold increased risk of cancer recurrence or cancer-related death (HR 2.71 [95% CI, 1.94-3.77], $p < 0.001$) (Table 3). The 5-year PFS for patients receiving a transfusion of RBCs was 26.2% (95% CI, 21.5-32.0) compared with 58.4% (95% CI, 49.0-69.6) in patients who were not transfused (Fig. 3). Furthermore, transfusion of RBCs was related with an increased risk of overall mortality (HR 2.87 [95% CI, 1.7-4.84], $p < 0.001$). In this regard, the 5-year OS for patients receiving transfusion of RBCs was 61.2% (95% CI, 55.4-67.7) compared with 84.1% (95% CI, 76.8-92.1) in patients who were not transfused (Fig. 4).

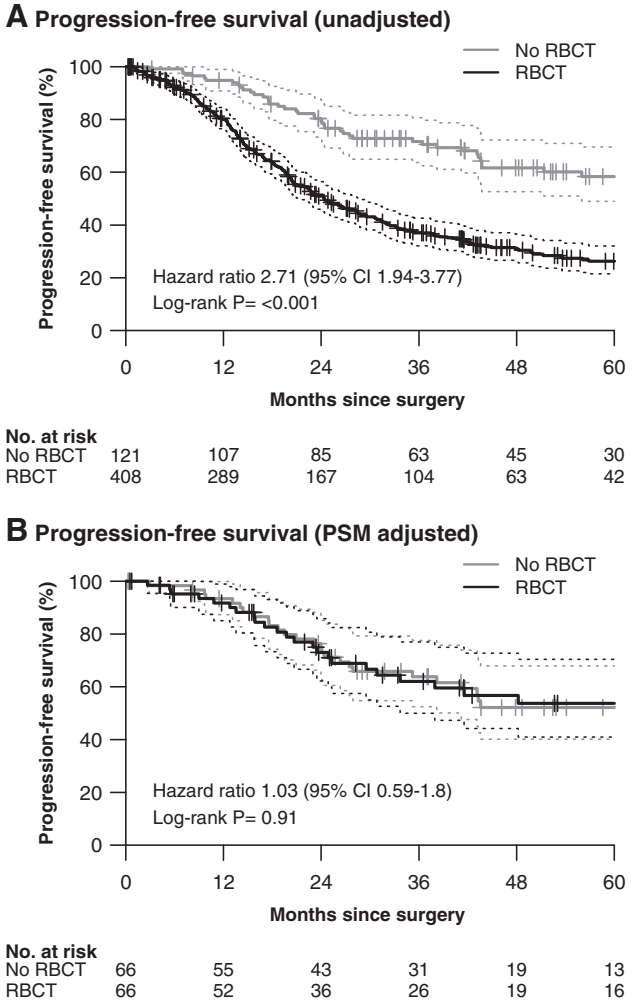

Fig. 3. PFS of patients not receiving and receiving perioperative transfusion of RBCs (RBCT) in unadjusted (A) and propensity score matching adjusted (B) analysis. Kaplan–Meier curves are shown with 95% confidence intervals and censored patients are indicated as tick marks. Hazard ratios with 95% confidence intervals are presented.

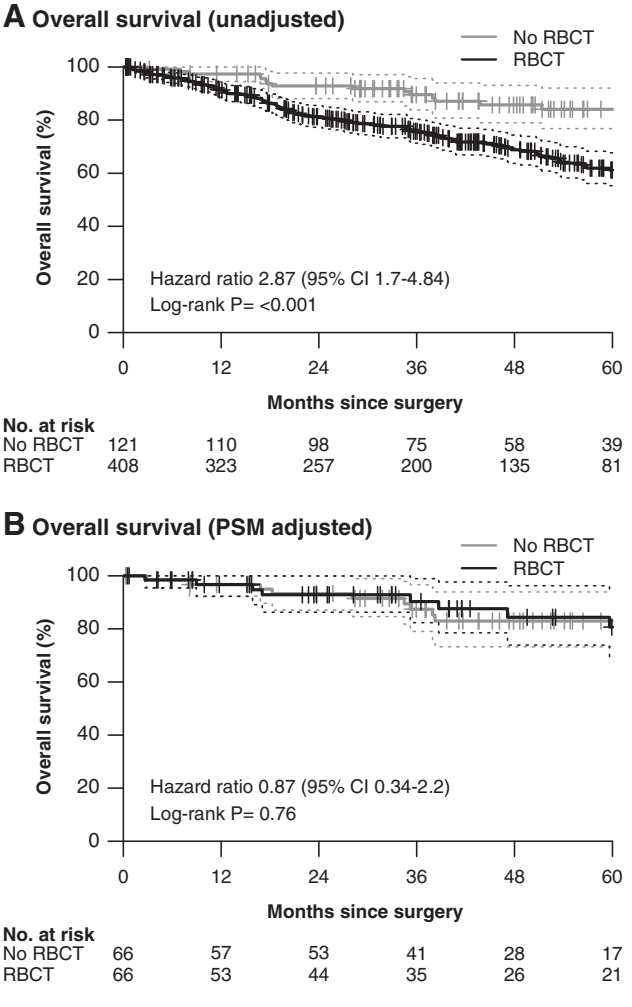

Fig. 4. OS of patients not receiving and receiving perioperative transfusion of RBCs (RBCT) in unadjusted (A) and propensity score matching adjusted (B) analysis. Kaplan–Meier curves are shown with 95% confidence intervals and censored patients are indicated as tick marks. Hazard ratios with 95% confidence intervals are presented.

After adjusting the analysis using multivariable CPH, transfusion of RBCs was not associated with an increased risk of cancer recurrence and cancer-related death (HR 1.26 [95% CI, 0.85-1.86], p = 0.23) (Table 3), or an increased risk of overall mortality (HR 1.31 [95% CI, 0.71-2.40], p = 0.37).

Applying a 1:1 pair PSM procedure, 66 pairs (55% of the maximum possible pairs) were identified, corresponding to a total of 132 patients in the matched cohort (Fig. 2). As indicated by the percentage of balance improvement of the PS, the bias of the prognostic determinants was reduced by 99.4%. In the matched cohort, prognostic determinants were well balanced between patients transfused with RBCs and patients who did not receive a transfusion of RBCs (PS 0.53 $\pm$ 0.23 vs. 0.53 $\pm$ 0.23, SMD 0.01, p = 0.94). In addition, no significant differences in other patient characteristics were found between the two groups (Tables 1 and 2). Transfused patients received a median of 2 RBC units (2; 3) per patient. The mean RBC storage duration of 23.6 ($\pm$8.3) days and the median follow-up time of 52.4 months (95% CI, 41.6-61.4) was comparable to the RBC storage duration and follow-up time of the entire study population. There was no difference in censoring between transfused and non-transfused patients regarding PFS and OS (p = 0.83, p = 0.82). In the matched cohort, transfusion of RBCs did neither increase the risk of cancer recurrence and cancer-related death (HR 1.03 [95% CI, 0.59-1.80], p = 0.91) (Table 3), nor did it increase the risk of overall mortality (HR 0.87 [95% CI, 0.34-2.2], p = 0.76). In this regard, the 5-year PFS for patients receiving transfusion of RBCs was 53.7% (95% CI, 40.9-70.5) compared with 52.2% (95% CI,

40.1-67.9) in patients who were not transfused (Fig. 3). The OS was 82.9% (95% CI, 73.2-93.9) in transfused patients and 80.7% (95% CI, 68.9-94.5) in non-transfused patients (Fig. 4).

DISCUSSION

This study demonstrates that a perioperative transfusion of RBCs at a hemoglobin threshold of 9 g/dL does not worsen oncological long-term outcome after surgery for primary epithelial ovarian cancer. There was a strong selection bias of prognostic determinants between patients who received a blood transfusion and patients who were not transfused, reflecting the medical circumstances that necessitate transfusion of RBCs. After controlling for between-group differences of prognostic determinants using two different and independent statistical approaches, perioperative transfusion of RBCs did not impact progression-free and overall survival.

In cancer surgery, the intra- and postoperative period represents a critical window for the development of a recurrent disease.[6,9] In this respect, transfusion of autologous RBCs has been shown to attenuate the NK cell–mediated cytolytic activity for up to 7 days after surgery.[4] Due to the potential of RBCs to impact the immunosurveillance of the recipient, we assessed if perioperative transfusion of RBCs increases the risk for a recurrent disease after curative surgery.

A median follow-up period of more than 50 months ensured that the primary endpoint could be sufficiently assessed. In this respect, the median PFS of 30 months was concordant with previous published cohorts with no residual disease after EOC surgery.[22] Similar to previous studies, many clinicopathological and surgical characteristics were strongly biased when patients who received a perioperative blood transfusion were compared with patients who were not transfused reflecting the different medical circumstances that necessitate transfusion of RBCs.[18–20] In this respect, the unadjusted analysis indicated that the transfusion of RBCs was associated with a 2.7-fold increased risk for cancer recurrence or death. However, after appropriate adjustment for prognostic determinants in EOC patients, PSM-adjusted as well as multivariable CPH-adjusted analysis revealed that transfusion of RBCs was no longer associated with an increased risk of cancer recurrence or death after curative surgery.

There are several conjectures why the transfusion of RBCs did not impact cancer outcome in our study. First, the transfusion of RBCs may not have altered the immunosurveillance of our patients. The decrease of NK-activity is primarily mediated by soluble white-blood-cell-derived mediators. While the impairment of NK function has been found after transfusion of non-leukoreduced RBCs,[4] there are currently no data available confirming this effect in prestorage, filter-leukoreduced RBCs, which are the predominant standard-of-care product today and have been used in the current study. A marked reduction in contaminating white blood cells and their soluble mediators might have prevented an alteration of NK activity in our patients transfused with leukoreduced RBCs. Second, the transfusion of RBCs did alter the immunosurveillance of the patients, but given a dose–response relationship, the dose of leukoreduced blood as represented by the units of transfused RBCs could have been insufficient for causing the immunosuppressive effects.[29] Third, although EOC is an "immunogenic tumor" that produces a spontaneous anticancer immune response detectable in peripheral blood, tissue, and ascites,[30] it has been shown that the tumor microenvironment of EOC itself may impair the functional capability of NK cells,[31] possibly making the effect size of transfusion of RBCs on the immunosurveillance insignificant. Fourth, the transfusion of RBCs did alter the immunosurveillance of the patients, but the effect is negligible compared to the cumulative effects of other perioperative determinants involved in the complex process of developing a recurrent disease. There is increasing evidence that perioperative factors can facilitate the development of a recurrent disease through numerous mechanisms.[32] These include deleterious processes due to perioperative unregulated paracrine, endocrine, immunological, and coagulation responses. In addition, surgery itself can cause a dysfunction of the immunosurveillance through a reduced anti-metastatic capacity of NK cells and CTLs that has been documented in both human patients[9,33,34] and animal models.[6,9,35]

Our findings are consistent with previous studies in EOC surgery[36] and major abdominal surgery for malignant tumors,[18–20,37–39] but are in contrast to a former study in EOC surgery[40] that demonstrated an increased risk for cancer recurrence and death if RBCs were transfused. This analysis, however, did not discriminate between patients with complete cytoreduction and patients with a macroscopic residual tumor <1 cm after surgery. Because complete cytoreduction has been shown to be of highest prognostic importance,[22] in the current study, only patients with complete cytoreduction were included to control for this important bias. In contrast to the findings of our study, a higher risk for cancer recurrence following perioperative transfusion of RBCs was found in recent meta-analyses of retrospective cohort studies that compared transfused patients with non-transfused patients in surgery of different cancer types.[13–17] However, evaluating the association of transfusion of RBCs with the risk for a recurrent disease in patients undergoing curative cancer surgery is challenging. Due to ethical considerations, randomized controlled trials in this field can only be conducted comparing transfusion of allogeneic versus autologous RBCs, non-leukoreduced versus leukoreduced RBCs, or liberal versus restrictive hemoglobin thresholds for transfusion. In this respect, data from retrospective cohort studies must consider the need for perioperative transfusion of RBCs due to patients'

comorbidities, perioperative complications, and disease severity. Because many of the included cohort studies lacked appropriately collecting prognostic determinants and controlling their effects on outcome, the results of the current available meta-analyses have to be interpreted carefully.

The current study has several limitations. The study design introduced a selection bias of prognostic determinants between transfused and non-transfused patients, because the medical circumstances that necessitate transfusion of RBCs and prognostic determinants are closely related. However, although it is impossible to completely eliminate this bias by statistical methods, the bias can be reduced to a minimum if the most important prognostic determinants are carefully defined, captured, and controlled. Therefore, the prognostic determinants of cancer recurrence in EOC were selected according to the latest evidence and two different and independent statistical analyses were performed to control for between-group differences. Although, we cannot rule out that further unknown confounders have affected the results, the analyses indicated a substantial bias reduction of the selected prognostic determinants. The high transfusion rate, the strong selection bias of prognostic determinants, and the greedy matching procedure resulted in a considerable "loss" of patients with high and low propensity scores. Therefore, many patients with high and low probabilities of receiving transfusion of RBCs were excluded from the analysis of the matched cohort. However, the distribution of the propensity scores in the matched cohort indicated that a relevant proportion of patients with high propensity scores could still be included into the analysis. In addition, because of the large fraction of patients that had to be excluded, there was a loss of statistical power in the matched cohort. Nonetheless, Kaplan–Meier curves were nearly identical so that it is unlikely that the loss of statistical power debilitated the validity to detect a significant difference between patients receiving a transfusion of RBCs and patients that were not transfused. So far, there is still limited evidence to suggest to which extent transfusion of RBC that were stored for prolonged intervals may increase the risk for recurrent disease after cancer surgery.[41,42] Finally, given a potential dose–response relationship between the number of transfused RBC units and the risk for a recurrent disease after cancer surgery,[29] a median of 4 units of RBCs in the study population, and 2 units of RBCs in the matched cohort might have been insufficient to cause immunosuppressive effects.

The studied patient cohort is one of the largest samples of EOC patients with complete cytoreduction after surgery. Accessing a prospectively maintained gynecological database to capture the most important prognostic determinants in ovarian cancer and using two different and independent statistical approaches allowed to evaluate a low-biased relationship between blood transfusions and oncologic outcomes.

In conclusion, this study demonstrates that a perioperative transfusion of RBCs at a hemoglobin threshold of 9 g/dL does not worsen oncological long-term outcome after surgery for primary EOC.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

Study concept, design of the study: O.H, A.F. Acquisition of data: O.H., S.G., O.M., I.B., A.P., J.S., A.F. Interpretation of data: O.H., S.G., J.A.G., W.B., I.B., J.S., A.F. Statistical analysis: A.K, O.H., A.F. Drafting of the manuscript: O.H., S.G., A.F. Critical revision of the manuscript for important intellectual content: All authors. Final revision of manuscript: O.H., J.A.G., W.B., A.F. Study supervision: A.F.

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