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Hematological Parameters in Endometrial Cancer; Worse Prognosis Reflecting Tumor Aggressiveness or Reduced Response to Radiotherapy?

Stephanie W Vrede[1,2]*; Hannah Donkers[3]; Casper Reijnen[4]; Anke Smits[1]; Nicole CM Visser[5,6]; Peggy M Geomini[7]; Huy Ngo[8]; Dennis van Hamont[9]; Brenda M Pijlman[10]; Maria Caroline Vos[11]; Marc P LM Snijders2; Roy Kruitwagen[12,14]; Ruud LM Bekkers[12,14]; Khadra Galaal[3]; Johanna MA Pijnenborg[1]

[1]Department of Obstetrics and Gynaecology, Radboud university medical centre, Nijmegen, the Netherlands.

[2]Department of Obstetrics and Gynaecology, Canisius-Wilhelmina Hospital, Nijmegen, the Netherlands.

[3]Department of Obstetrics and Gynaecology, Royal Cornwall Hospital NHS Trust, Truro, Cornwall, United Kingdom.

[4]Department of Radiation Oncology, Radboud university medical centre, Nijmegen, the Netherlands.

[5]Department of Pathology, Stichting PAMM, Eindhoven, the Netherlands.

[6]Department of Pathology, Radboud University Medical Centre.

[7]Department of Obstetrics and Gynaecology, Maxima Medical Centre, Veldhoven, the Netherlands.

[8]Department of Obstetrics and Gynaecology, Elkerliek Hospital, Helmond, the Netherlands.

[9]Department of Obstetrics and Gynaecology, Amphia Hospital, Breda, the Netherlands

[10]Department of Obstetrics and Gynaecology, Jeroen Bosch Hospital, 's-Hertogenbosch, the Netherlands.

[11]Department of Obstetrics and Gynaecology, ElisabethTweesteden Hospital, Tilburg, the Netherlands.

[12]Department of Obstetrics and Gynaecology, Maastricht UMC+, Maastricht, the Netherlands.

[13]Department of Obstetrics and Gynaecology, Catharina Hospital, Eindhoven, the Netherlands.

[14]Maastricht University, Grow - School for Oncology and Reproduction, Maastricht, the Netherlands.

Abstract

Objective: In Endometrial Cancer (EC), preoperative anemia, thrombocytosis and leukocytosis appear to be associated with worse prognosis. It remains unclear whether these parameters solely reflect tumor aggressiveness, or also impact response to adjuvant treatment. Therefore, our primary aim is to evaluate the prognostic relevance of anemia, thrombocytosis and leukocytosis on survival in EC. Secondary, to explore their predictive relevance in response to radiotherapy in EC.

Material and methods: A retrospective multicenter cohort study was performed within 10 hospitals. Preoperative hematological parameters were defined as: Anemia – hemoglobin <7.45 mmol/L (<12 g/Dl), thrombocytosis – platelets >400 x 10^9 platelets/L, leukocytosis – leukocytes >10 x 10^9/L. The relationship

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Correspondance: *Stephanie W Vrede, Department of Obstetrics and Gynaecology, Radboud University Medical Centre Geert Grooteplein 10, P.O. Box 9101, 6500 HB, Nijmegen, the Netherlands.*

Tel: +31618141496; Email: stephanie.vrede@radboudumc.nl

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of hematological parameters with clinicopathological characteristics, ESGO/ESTRO/ESP risk groups and survival were evaluated. Furthermore, the predictive value of hematological parameters was determined on the overall response to adjuvant radiotherapy and for the ESGO/ESTRO/ESP intermediate-risk group solely receiving radiotherapy.

Results: A total of 894 patients were included with a median follow-up of 4.5 years. Anemia was present in 103 (11.5%), thrombocytosis in 79 (8.8%) and leukocytosis in 114 (12.7%) patients. The presence of anemia or thrombocytosis was significantly associated with ESGO/ESTRO/ESP high-risk (respectively, *P*=0.002 and *P*=0.041). In the entire cohort, anemia remained independently associated with decreased disease-specific survival (DSS) (HR 2.31, 95% CI (1.19-4.50), *P*=0.013) after adjusting for age, the abnormal hematological parameters and ESGO/ESTRO/ESP risk groups. In patients that were treated with adjuvant radiotherapy (n=239), anemia was associated with significant reduced 5-year DSS and recurrence-free survival (*P*=0.005 and *P*=0.025, respectively). In ESGO/ESTRO/ESP intermediate risk patients that received solely vaginal brachytherapy (n=74), anemia was associated with reduced DSS (*P*=0.041).

Conclusions: Current data demonstrate the importance of preoperative anemia as independent prognostic factor in patients with EC. Moreover, anemia seems to be associated with reduced response to radiotherapy. Prospective validation in a larger study cohort is needed to verify anemia as predictive biomarker for radiotherapy.

Keywords: Anemia; Prognostic; Predictive; Radiotherapy; Vaginal brachytherapy.

Abbreviations: EC: Endometrial Cancer; ESGO/ESTRO/ESP: European Society of Gynaecological Oncology; European Society For Radiotherapy and Oncology; European Society of Pathology; FIGO: International Federation of Gynecology and Obstetrics; LVSI: Lymphovascular Space Invasion; RT: Radiotherapy; UK: United Kingdom; VBT: Vaginal Brachytherapy; OR: Odds Ratio; CI: Confidence Interval; HR: Hazard Ratio; DSS: Disease-Specific Survival.

Introduction

Endometrial cancer (EC) is the most common gynecologic malignancy in industrialized countries with incidence rates rising due to aging and obesity. Most patients are diagnosed with low-grade EC (grade 1-2 endometrioid EC), and generally have a favorable prognosis [1]. Around 20% of patients are diagnosed with high-grade EC (grade 3 endometrioid EC and non-endometrioid EC), have an overall poor prognosis and are associated with an increased risk of regional or distant metastases [1]. Currently, primary surgical treatment is based on preoperative tumor grade and histology.

According to the recent ESGO/ESTRO/ESP (European Society of Gynaecological Oncology – European SocieTy for Radiotherapy and Oncology – European Society of Pathology) guideline, adjuvant treatment is based on risk classification groups incorporating FIGO (Federation International of Gynecology and Obstetrics) stage, tumor grade and histology, Lymphovascular Space Invasion (LVSI) and with or without molecular markers [2]. Often routinely obtained preoperative clinical biomarkers, such as hematological parameters, may contribute to identification of patients with extended disease and/or aggressive tumor behavior that might respond differently to adjuvant therapy [3-5].

Endometrial carcinogenesis is characterized by chronic inflammation with elevated pro-inflammatory cytokines and acute phase proteins [6]. Overexpression of inflammatory cytokines could contribute to the development of cancer-related anemia, thrombocytosis and leukocytosis, and could generate a protumorigenic environment [7-10]. Preoperative abnormal hematological parameters like anemia, thrombocytosis and/or leukocytosis, have been shown to be associated with FIGO advanced-stage and unfavorable outcome, however results remain conflicting [8,9,11-16].

Several studies showed an adverse impact of anemia to Radio Therapy (RT) response in solid tumors, explained by the fact that anemia is proposed to be a surrogate maker for tumor hypoxia [4,17]. Hypoxia is very common in solid tumors and leads to cellular stress response, which allows tumor cells to survive. In addition, these hypoxic conditions may also protect tumor cells from downstream DNA breaks and lethality induced by radiotherapy [18,19]. Within gynecological tumors, leukocytosis was also observed to have an adverse predictive impact on RT response [5]. So far, no studies reported the impact of thrombocytosis on RT in solid tumors.

Based on conflicting results in outcome of abnormal preoperative hematological parameters in endometrial cancer, we aim to evaluate the prognostic relevance of anemia, thrombocytosis and leukocytosis on survival. Second, we aim to explore the predictive relevance of these abnormal hematological parameters on response to adjuvant RT. We hypothesize that patients with anemia and thrombocytosis have reduced survival due to advanced stage and/or disseminated EC, and anemia might have negative impact on response to adjuvant RT.

Material and methods

Study cohort

A multicenter cohort study was performed with a combination of prospective and retrospectively collected data in patients diagnosed with EC. This study is a collaboration between the Netherlands and the United Kingdom (UK) by which data of nine hospitals in the Netherlands (PIpelle Prospective ENDO metrial carcinoma (PIPENDO) cohort) [20] and one in the UK [21] were merged. The design and patient cohort of both cohorts, including 946 patients in total (PIPENDO and UK), have been published previously [20,21]. A study flowchart is shown in the Figure S1.

Data collection

All patients were surgically treated between 2006-2015. For the Dutch participating hospitals patient characteristics, postoperative tumor histology, grade and FIGO staging were collected prospectively [20]. Preoperative hemoglobin level, platelet- and leukocyte counts were collected retrospectively from hospital records. For the UK center, all clinicopathological characteristics and preoperative hematological parameters were collected retrospectively [21]. Regarding the data collection of nodal status, in the Netherlands and UK surgical staging is selectively performed in patients with preoperative high-grade histology (grade 3 endometrioid EC and non-endometrioid EC) and in case of clinical suspicion of extended disease, according to the Dutch and British EC guideline [22,23].

The sole additional inclusion criteria used for this study was that patients were only included if at least one of the three preoperative hematological parameters was conducted ≤6 weeks prior to surgery, resulting in 896 patients.

Statistical analysis

The hematological parameters were analyzed as a dichotomous value, with defined cut-offs. Anemia was defined according to the World Health Organization as hemoglobin level <7.45 mmol/L (<12 g/Dl) [24]. Thrombocytosis as platelet counts >400 x 10^9 /L according multiple studies involving gynecologic malignancies[8] and leukocytosis as leukocyte counts >10 x 10^9/L [10].

The risk classification groups were classified according to the ESGO/ESTRO/ESP guideline; low, intermediate, high-intermediate, high and advanced/metastatic risk group [2]. To explore the response on RT, all patients who received solely adjuvant RT were included for the second analysis. To further refine response of RT and in order to prevent treatment bias by including patients who were not treated according to the recent guideline, patients only classified as ESGO/ESTRO/ESP intermediate risk were included (flowchart secondary analysis Figure S2). According to the guideline, these patients are recommended to receive adjuvant Vaginal Brachytherapy (VBT) [2]. Whereas other risk classification groups include observation or combined chemoradiotherapy.

For statistical analyses, Statistical Package for the Social Sciences, version 25.0 (IBM, New York, NY, USA) was applied. The results were considered significant with *P*-value less than 0.05 (*P*<0.05). Clinicopathological characteristics between dichotomous hematological subgroups were compared using the χ^2 or Fisher's exact test for categorical data, and the non-parametric Mann-Whitney U-test for continuous variables. Association between exposure and outcome are shown as Odds Ratio (OR), 95% Confidence Interval (CI) and *P*-value. Survival analyses were performed using Kaplan-Meier curves and univariable and multivariable Cox-regression. Associations are shown as Hazard Ratio (HR), 95% CI and *P*-value. Disease-Specific Survival (DSS) was defined as time from date of diagnosis to date of death by EC and Recurrence-Free Survival (RFS) was defined as time from surgery to time of recurrence from EC disease, all censored by date of last contact.

Results

Patients

A total of 896 EC patients were included with a least one hematological parameter. Two patients had abnormally high leukocyte count (>50 x 10^9/L) due to chronic lymphatic leukemia and unknown cause, these patients were excluded, resulting in 894 EC patients (54.8% British and 45.2% Dutch) included in this study with a median follow-up of 4.5 years (range 0-10 years) (Figure S1). Clinicopathological characteristics of the study cohort are shown in Table 1. Median age was 65.9 (27.2-93.8) years and median body mass index 29.7 (16.4-60.9) kg/m^2. Of 653 (73.0%) EC patients all three hematological parameters were available. Median preoperative hemoglobin level was 8.4 mmol/L, median platelet count 298.3 x 10^9 platelets/L and median leukocyte count 8.1 x 10^9/L. Anemia was present in 103 (11.3%), thrombocytosis in 79 (8.6%) and leukocytosis in 114 patients (12.5%). Most patients were diagnosed with low-grade (grade 1-2), FIGO stage I-II and endometrioid EC (respectively, 69.4%, 90.2% and 82.2%). Lymphadenectomy was performed in 205 patients (22.9%) of whom 34 (16.5%) had lymph node metastasis. Adjuvant treatment was administered in 344 patients (38.5%). A total of 239 patients (69.5%) received RT of which 132 patients (55.2%) VBT and 107 patients (44.8%) external beam radiation therapy with or without VBT. Hundred and twenty-four patients (13.9%) developed recurrent EC, and 160 patients (17.9%) were deceased of which 99 (61.8%) deaths were directly related to EC.

Preoperative hemoglobin-, platelet- and leukocyte level in relation to clinicopathological characteristics are shown in Table 2. Hemoglobin level was measured in 894 (100.0%), platelet count in 721 (80.6%) and leukocyte count in 667 patients (74.6%). Patients with anemia were significantly associated with grade 3 EC (OR 1.81, 95% CI 1.18-2.79), LVSI (OR 1.61, 95% CI 1.00-2.57), and ESGO/ESTRO/ESP high risk (OR 2.11, 95% CI 1.30-3.42). The presence of thrombocytosis was significantly associated with LVSI (OR 1.77, 95% CI 1.04-2.99), and ESGO/ESTRO/ESP high risk (OR 1.78, 95% CI 1.02-3.11). Leukocytosis was significantly associated with ESGO/ESTRO/ESP advanced/metastatic risk (OR 2.72, 95% CI 1.06-6.97).

Outcome

The 5-year DSS and RFS of preoperative anemia, thrombocytosis and leukocytosis are shown in Figure 1A-F. Patients with anemia had a significant reduced 5-year DSS and RFS compared to patients with normal hemoglobin level (respectively, *P*<0.001 and *P*<0.001) (Figure 1A,1D). Patients with thrombocytosis showed significant reduced 5-year DSS compared to normal platelet count (*P*=0.023), no difference was found for RFS (Figure 1B,1E). For patients with leukocytosis compared with normal leukocyte count, no significant difference in DSS and RFS was found (Figure 1C,1F).

In multivariable analysis after adjusting for age, the three abnormal hematological parameters and the ESGO/ESTRO/ESP risk groups, only anemia, age and ESGO/ESTRO/ESP high- and advanced/metastatic risk groups remained independently associated with a reduced DSS. None of the hematological parameters were independently associated with a decreased RFS (Table 3).

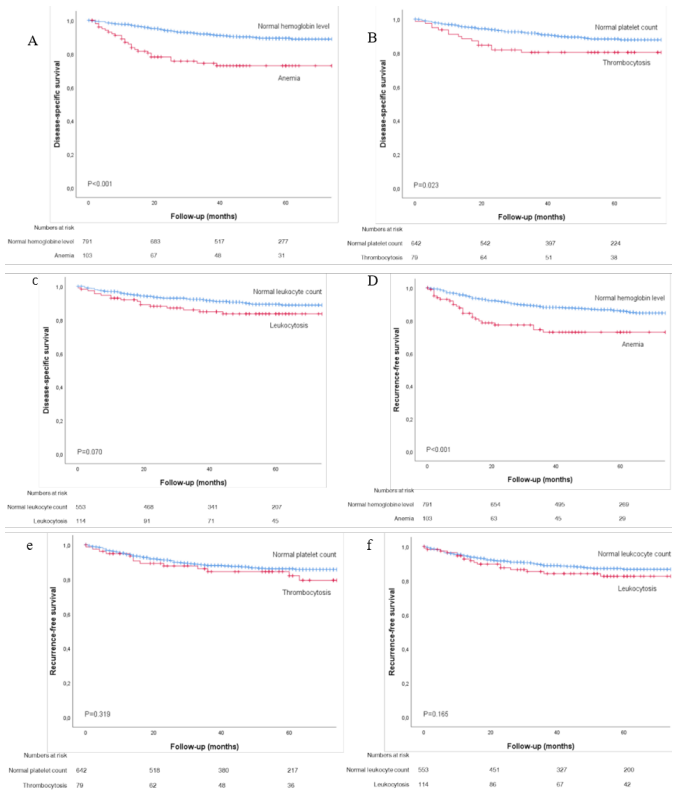

Figure 1 A-F: 5-year disease-specific survival (DSS) and recurrence-free survival (RFS) of patients with normal and abnormal hematological parameters. **A**. 5-year DSS of patients with and without anemia. **B**. 5-year DSS of patients with and without thrombocytosis. **C**. 5-year DSS of patients with and without leukocytosis. **D**. 5-year RFS of patients with and without anemia. **E**. 5-year RFS of patients with and without thrombocytosis. **F**. 5-year RFS of patients with and without leukocytosis.

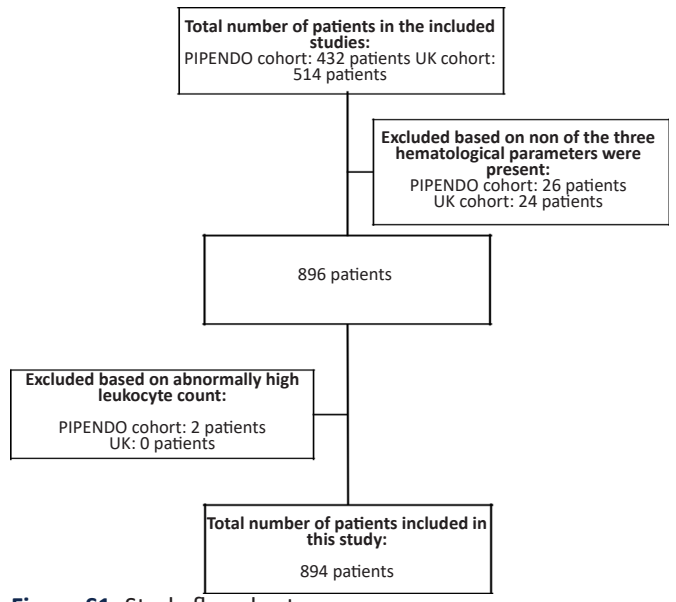

Figure S1: Study flowchart

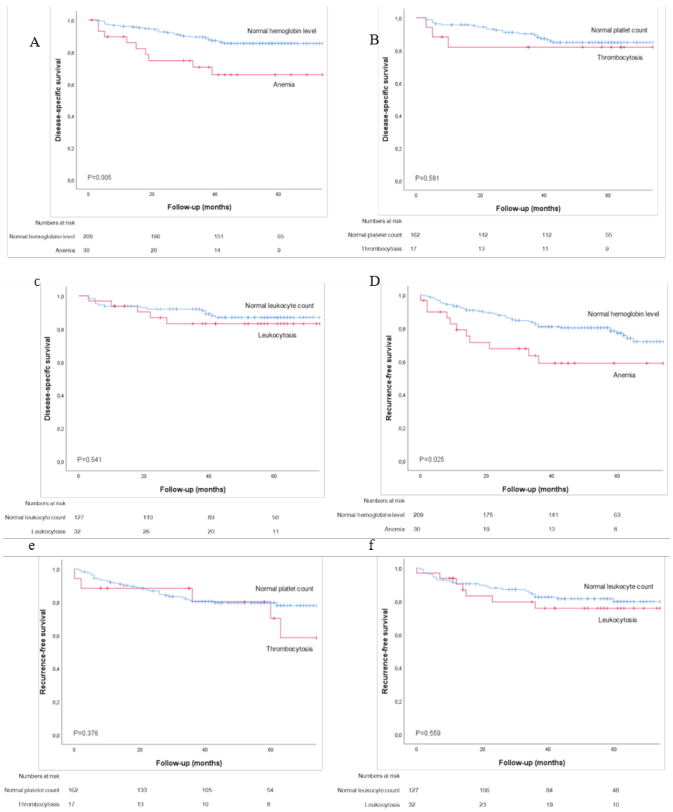

Figure 2 A-F: 5-year disease-specific survival (DSS) and recurrence-free survival (RFS) of patients with normal and abnormal hematological parameters within patients with solely adjuvant radiotherapy (RT). **A**. 5-year DSS of patients with and without anemia in patient with adjuvant RT. **B**. 5-year DSS of patients with and without thrombocytosis in patients with adjuvant RT. **C**. 5-year DSS of patients with and without leukocytosis in patients with adjuvant RT. **D**. 5-year RFS of patients with and without anemia with adjuvant RT. **E**. 5-year RFS of patients with and without thrombocytosis with adjuvant RT. **F**. 5-year RFS of patients with and without leukocytosis with adjuvant RT.

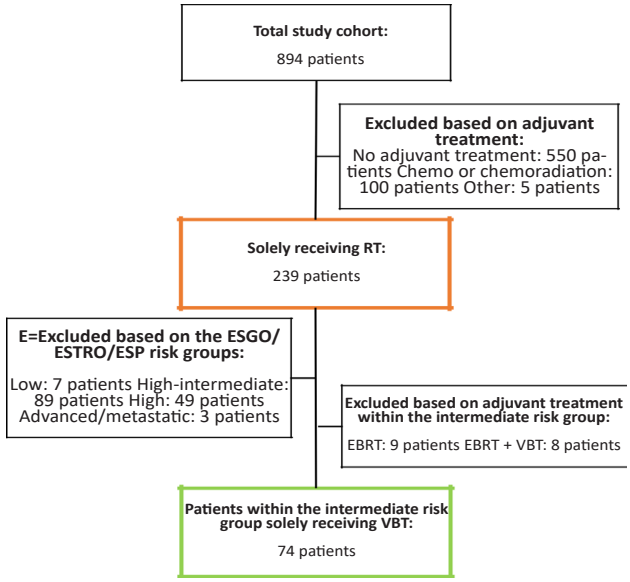

Figure S2: Flowchart secondary analysis

Table 1: Baseline clinic pathological characteristics.

Patient characteristics		Total (n=894)
Age (years)		65.9 (27.2-93.8)
BMI (kg/m^2)		29.7 (16.4-60.9)
Serum values		
Hemoglobin mmol/L		8.4 (3.9-10.6)
Hemoglobin <7.45 mmol/L		103 (11.3)
Platelets x 10^9 /L		298.3 (13.9-781.0)
Platelets >400 x 10^9		79 (8.6)
Leukocytes x 10^9/L		8.1 (2.2-33.5)
pLeukocytes >10 x 109/L		114 (12.5)
Final tumor histology		
Tumor Grade	1-2	620 (69.4)
	3	274 (30.6)
Histology	Endometrioid	735 (82.2)
	Non-endometrioid	159 (17.8)
LVSI	Yes	177 (19.8)
	No	717 (80.2)
FIGO stage	Early (I-II)	806 (90.2)
	Advanced (III-IV)	88 (9.8)
Lymph node status	Positive (N1)	34 (3.8)
	Negative (N0)	171 (19.1)
	Unknown† (Nx)	689 (77.1)
ESGO/ESTRO/ESP risk groups	Low	409 (45.7)
	Intermediate	159 (17.8)
	High-intermediate	162 (18.1)
	High	140 (15.7)
	Advanced/metastatic	24 (2.7)
Adjuvant treatment		
None		550 (61.5)
RT	VBT	132 (14.8)
	EBRT (+/- VBT)	107 (11.9)
CT+CRT		100 (11.2)
Other		5 (0.6)
Outcome		
Recurrence	Yes	124 (13.9)
	No	770 (86.1)
Mortality	Overall	160 (17.9)
	EC-related	99 (11.1)

Data is presented in numbers (%) or median (IQR).

Abbreviations: n: Number; FIGO: Federation International Gynecology Obstetric; ESGO: European Society of Gynaecological Oncology; ESTRO: European Society for Radiotherapy and Oncology; ESP: European Society of Pathology; RT: Radiotherapy; VBT: Vaginal Brachytherapy; EBRT: External beam radiation therapy; CT: Chemotherapy; CRT: Chemoradiation; EC: Endometrial Cancer. †no lymphadenectomy performed.

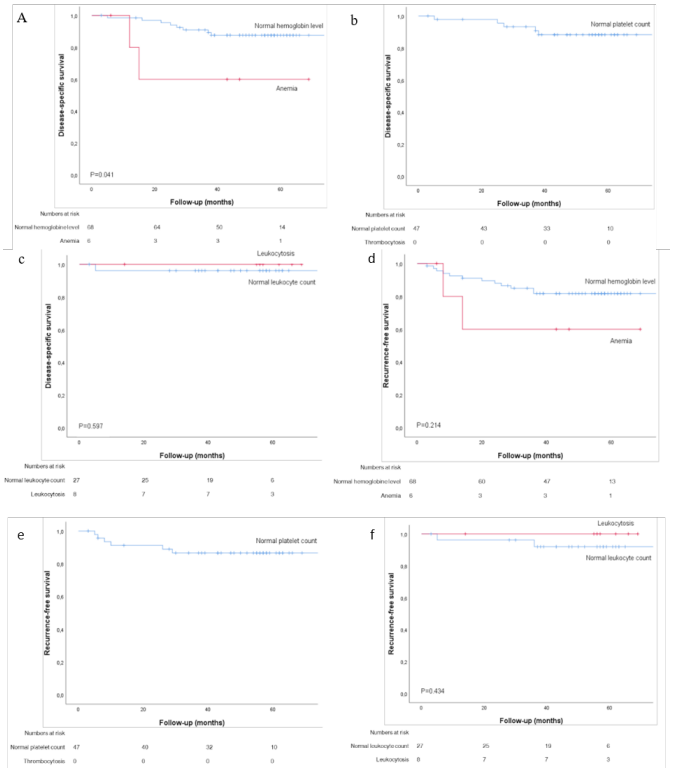

Figure S3 A-F: 5-year Disease-Specific Survival (DSS) and Recurrence-Free Survival (RFS) of patients with normal and abnormal hematological parameters within patients classified as intermediate risk who received VBT. **A**. 5-year DSS of patients with intermediate risk and VBT, and with and without anemia. **B**. 5-year DSS of patients patients with intermediate risk and VBT, and with and without thrombocytosis. **C**. 5-year DSS of patients with intermediate risk and VBT, and with and without leukocytosis. **D**. 5-year RFS of patients with intermediate risk and VBT, and with and without anemia. **E**. 5-year RFS of patients with intermediate risk and VBT, and with and without thrombocytosis. **F**. 5year RFS of patients with intermediate risk and VBT, and with and without leukocytosis.

Impact of hematological parameters on response to radiotherapy

The 5-year DSS and RFS of the preoperative hematological parameters in all patients who received solely adjuvant RT are shown in Figure 2A-F. Anemia was associated with a significant reduced DSS and RFS compared to normal hemoglobin level (respectively, *P*=0.005 and *P*=0.025) (Figure 2A,2D). Thrombocytosis and leukocytosis did not significantly impact the response to RT (Figure 2B,2C,2E,2F). The 5-year DSS and RFS of the hematological parameters within patients classified as ESGO/ESTRO/ESP intermediate risk who received solely VBT are shown in Figure S3A-E**.** Patients with anemia had a significant decreased DSS compared to normal hemoglobin level (*P*=0.041). No significant difference in DSS and RFS were found for patients with thrombocytosis or leukocytosis, however numbers were low.

Table 2: Clinicopathological characteristics in relation to hemoglobin-, leukocytes- and thrombocytosis-level.

hemoglobin (n=103) (n=791)		Normal	Anemia	*P*	Normal platelets (n=642)	Thrombocytosis (n=79)	*P*	Normal leukocytes (n=553)	Leukocytosis (n=114)	*P*
Patient characteristics										
Age		65.7 (27.2- 91.0)	68.2 (33.8-93.8)	0.246	66.0 (31.2-93.8)	64.0 (27.2-90.7)	**0.017***	66.0 (31.2-93.8)	65.0 (27.2-86.0)	0.386
Final tumor histology										
Tumor grade	1-2	560 (70.8)	60 (58.3)	**0.009***	448 (69.8)	52 (65.8)	0.471	383 (69.3)	81 (71.1)	0.705
	3	231 (29.2)	43 (41.7)		194 (30.2)	27 (34.2)		170 (30.7)	33 (28.9)	
Endometrioid		656 (82.9)	79 (76.7)	0.120	531 (82.7)	62 (78.5)	0.353	455 (81.7)	94 (82.5)	0.856
Non-endometrioid		135 (17.1)	24 (23.3)		111 (17.3)	17 (21.5)		101 (18.3)	20 (17.5)	
LVSI	Yes	149 (18.8)	28 (27.2)	**0.046***	121 (18.8)	23 (29.1)	**0.031***	109 (19.7)	26 (22.8)	0.454
	No	642 (81.2)	75 (72.8)		521 (81.2)	56 (70.9)		444 (80.3)	88 (77.2)	
ESGO/ESTRO/ESP risk groups										
Low risk		372 (47.0)	37 (35.9)	**0.033***	309 (48.1)	31 (39.2)	0.135	270 (48.8)	53 (46.5)	0.650
Intermediate risk		146 (18.5)	12 (11.7)	0.088	105 (16.4)	7 (8.9)	0.083	77 (13.9)	17 (14.9)	0.782
High-intermediate risk		140 (17.7)	22 (21.4)	0.364	114 (17.8)	17 (21.5)	0.413	101 (18.3)	17 (14.9)	0.393
High risk		114 (14.4)	27 (26.2)	**0.002***	97 (15.1)	19 (24.1)	**0.041***	92 (16.6)	20 (17.5)	0.813
Advanced/metastatic		19 (2.4)	5 (4.9)	0.148	17 (2.6)	5 (6.3)	0.073	13 (2.4)	7 (6.2)	**0.031***
Adjuvant treatment										
None		499 (63.1)	51 (49.5)	**0.009***	412 (64.2)	42 (53.2)	0.066	361 (65.2)	64 (56.1)	0.069
RT	VBT	122 (15.4)	10 (9.7)	0.124	87 (13.6)	3 (3.8)	**0.013***	58 (10.5)	15 (13.2)	0.406
	EBRT (+/- VBT)	87 (11.0)	20 (19.4)	**0.012***	75 (11.6)	14 (17.7)	0.124	69 (12.5)	17 (14.9)	0.480
CT+CRT		79 (10.0)	21 (20.4)	**0.002***	65 (10.1)	19 (24.0)	**<0.001***	62 (11.3)	17 (15.0)	0.260
Other		4 (0.5)	1 (1.0)	0.459	3 (0.5)	1 (1.3)	0.372	3 (0.5)	1 (0.8)	0.528

Data is presented in numbers (%), median (range)

Abbreviations: n: number; LVSI: Lymphovascular Space Invasion; ESGO: European Society of Gynaecological Onoclogy; ESTRO: European Society for Radiotherapy and Oncology; ESP: European Society of Pathology; RT: Radiotherapy; VBT: Vaginal brachytherapy; EBRT: External beam radiation therapy; CT: Chemotherapy; CRT: Chemoradiation * P<0.05.

Table 3: Cox regression univariable and multivariable analysis of Disease-Specific Survival (DSS) and Recurrence-Free Survival (RFS).

Variable	Univariable DSS		Multivariable DSS Event 66		Univariable RFS		Multivariable RFS Event 78	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Patient characteristics								
Age (continuous)	1.04 (1.02-1.06)	<0.001*	1.03 (1.00-1.06)	0.009*	1.04 (1.01-1.05)	<0.001*	1.03 (1.00-1.05)	0.022*
Hematological parameters								
Anemia	3.19 (2.02-5.02)	<0.001*	2.31 (1.19-4.50)	0.013*	2.32 (1.49-3.60)	<0.001*	1.71 (0.91-3.19)	0.091
Thrombocytosis	1.90 (1.08-3.34)	0.025*	1.06 (0.49-2.30)	0.872	1.31 (0.73-2.36)	0.363	0.70 (0.32-1.55)	0.382
Leukocytosis	1.65 (0.95-2.86)	0.074	1.37 (0.74-2.55)	0.312	1.45 (0.85-2.44)	0.168	1.53 (0.84-2.74)	0.159
ESGO/ESTRO/ESP risk groups								
Low	1		1		1		1	
Intermediate	7.59 (3.01-19.12)	<0.001*	3.06 (0.98-9.53)	0.053	6.20 (3.06-12.55)	<0.001*	2.38 (0.88-6.39)	0.087
High-intermediate	3.90 (1.38-10.96)	0.010*	1.59 (0.44-5.65)	0.472	5.23 (2.56-10.89)	<0.001*	3.43 (1.44-8.16)	0.005*
High	32.66 (13.99-76.23)	<0.001*	18.3 (7.64-43.94)	<0.001*	22.21 (11.64-42.37)	<0.001*	16.78 (8.05-34.83)	<0.001*
Advanced/metastatic 101.97 (40.33-257.79)		<0.001*	72.1 (27.36-189.97)	<0.001*	36.59 (15.79-84.74)	<0.001*	33.68 (13.15-86.29)	<0.001*

Abbreviations: DDS: Disease-specific survival; RFS: Recurrence-free survival; HR: Hazard ratio; CI: Confidence interval; ESGO: European Society of Gynaecological Onoclogy; ESTRO: European Society for Radiotherapy and Oncology; ESP: European Society of Pathology, * *P*<0.05.

Discussion

In this study, the prognostic and predictive relevance of preoperative abnormal hematological parameters in patients with EC was evaluated. Anemia was identified as an independent prognostic factor for DSS, along with age and ESGO/ESTRO/ESP 'high- and advanced/metastatic' risk. Furthermore, anemia seemed an overall predictive factor for response to adjuvant RT, and specifically for patients with ESGO/ESTRO/ESP intermediate risk who received solely VBT.

Although most patients with EC present with postmenopausal bleeding as an early symptom, this rarely causes anemia at diagnosis. Hence, the development of cancer-related anemia in EC is more likely caused by inflammatory cytokines which results in a shortened survival of red blood cells, suppression of erythroid progenitor cells, impaired iron utilization, and inadequate Erythropoietin (EPO) production [7,25]. Anemia in patients with an absolute or relative EPO deficiency seems to be more aggressive in solid tumors [26]. Therefore, it is suggested that preoperative anemia in EC could be a biomarker of tumor burden and/or aggressive tumor behavior [25,26]. In our study cohort we observed that patients with anemia were significantly more often allocated to ESGO/ESTRO/ESP high risk group, grade 3 EC, and the presence of LVSI. In both univariable and multivariable DSS analysis, we found anemia as independent prognostic factor. To our knowledge, the presence of anemia has so far not been related to the ESGO/ESTRO/ESP risk groups. Previous studies did show a significantly higher prevalence of anemia in patients classified into the ESGO/ESTRO/ESP high risk group; FIGO advanced-stage, grade 3 EC and LVSI [16]. The 5-year RFS was significantly reduced in patients with anemia compared to those without anemia. However, anemia was not an independent prognostic factor for the RFS, comparable to the findings of Wilairat et al [27].

Cancer-related anemia may also cause tumor hypoxia, which may lead to a reduced response to RT [4,17-19]. Normally, hypoxia will lead to an EPO increase, however due to the cancer-associated inflammation the EPO production is insufficient and the iron metabolism is impaired. VBT is given for local control of the tumor and EBRT could be applied to control locoregional recurrence [19]. In patients within our study, who received RT and even with solely VBT within the ESGO/ESTRO/ESP intermediate risk group, anemia was correlated with a significantly reduced DSS. However, numbers were low and therefore multivariable analysis was not achievable. So far, no other studies including EC patients have been performed to compare our findings.

Three recent meta-analyses published the clinicopathological and/or prognostic significance of preoperative thrombocytosis in EC [8,9,13]. In line with our findings, a significant association of thrombocytosis with FIGO advanced-stage, LVSI and grade 2-3 EC was found [8,13]. The prognostic relevance, however, still remains conflicting in EC studies, probably due to different used cut-off values for thrombocytosis [8,9,13]. Comparable to our study, Njolstad et al. found a significant reduced DSS of patients with thrombocytosis [11]. However, thrombocytosis as dichotomous value instead of continuous platelet count was not found as independent factor for DSS and RFS [8]. The pathophysiological mechanism between tumor behavior and preoperative thrombocytosis is not fully elucidated [13]. The overexpression of inflammatory cytokines results in an increase of megakaryocyte maturation which causes increased platelet production [28]. Some hypothesize that platelets infiltrate tumor tissue and contribute to tumor growth by secreting pro-angiogenic factors and pro-tumorigenic factors, while others suggest a plateletcancer interaction facilitating cancer cell migration, which contributes cancer metastasis [29].

The impact of leukocytosis on tumor behavior may also be explained by upregulation of inflammatory cytokines and hematopoietic growth factor through tumor cells, thus promoting enhanced inflammation, leukocytosis, angiogenesis and tumor

cell proliferation [6,30]. We observed a significant association between leukocytosis and the ESGO/ESTRO/ESP advanced/metastatic risk group in our study cohort, however leukocytosis was not significant in univariable and multivariable analysis. A recent meta-analysis found a correlation between leukocytosis and FIGO advanced-stage [15], of whom only one study performed a multivariable analysis for RFS with comparable results as our study [14].

Due to the pro-angiogenic factors induced with elevated platelet and leukocyte count, its suspected that angiogenesis will lead to a better drug or oxygen access to tumor cells, however there is a lack of homogeneity of vasculature density in different parts of the same tumor which could affect outcome and response to adjuvant treatment [4]. Although we did not observe impact of thrombocytosis and/or leukocytosis on response to RT, included numbers were low. In patients with cervical cancer leukocytosis was related to poor response to RT, but due to differences in carcinogenesis it may be difficult to compare those results with EC [5].

There are some limitations inherent to the retrospective design. First, adjuvant treatment was not uniformly applied which could lead to differences in outcome. Second, due to the fact that most of our labs do not run routine complete blood count, platelet- and leukocyte count were not available for all included patients. Finally, complete molecular data according The Cancer Genome Atlas is not available for the patients in this cohort. However, within a subset of the PIPENDO cohort, we do have immunohistochemistry of p53 and mismatch repair proteins. Within patients with p53-abnormal, anemia was associated with significant reduced DSS and RFS compared to patients with normal hemoglobin (data not shown).

To our knowledge, this is the first study that addressed the relationship of all three, often routinely obtained, preoperative abnormal hematological parameters with clinicopathological characteristics and univariable and multivariable outcome in EC. Other strengths of this study includes its multicenter design resulting in the largest patient cohort to date, and a well-documented and long follow-up period.

Future studies in a prospective study design, may determine the prognostic and/or predictive value of preoperative abnormal hematological markers (more specific anemia) in addition to the molecular markers in EC. When confirmed, studies should explore in more detail the cause between for example anemia and impaired prognosis.

Conclusion

Our data demonstrated the independent prognostic impact of preoperative anemia in patients with EC. In addition, anemia seems to be associated as predictive biomarker for response to radiotherapy. It remains unclear whether preoperative anemia reflects tumor aggressiveness or reduced response to radiotherapy. So, prospective validation in a larger study cohort is needed to verify anemia as predictive biomarker for radiotherapy.

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References

1. Bokhman JV. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983; 15: 10-17.
2. Concin N, Matias-Guiu X, Vergote I, Cibula D, Mirza MR, et al. ESGO/ESTRO/ESP guidelines for the management of patients with endometrial carcinoma. Int J Gynecol Cancer. 2021; 31: 12-39.
3. Reijnen C, Gogou E, Visser NCM, Engerud H, Ramjith J, et al. Preoperative risk stratification in endometrial cancer (ENDORISK) by a Bayesian network model: A development and validation study. PLoS Med. 2020; 17: e1003111.
4. Koukourakis MI, Giatromanolaki A, Sivridis E, Fezoulidis I. Cancer vascularization: implications in radiotherapy? Int J Radiat Oncol Biol Phys. 2000; 48: 545-553.
5. Cho Y, Kim KH, Yoon HI, Kim GE, Kim YB. Tumor-related leukocytosis is associated with poor radiation response and clinical outcome in uterine cervical cancer patients. Ann Oncol. 2016; 27: 2067-2074.
6. Modugno F, Ness RB, Chen C, Weiss NS. Inflammation and endometrial cancer: a hypothesis. Cancer Epidemiol Biomarkers Prev. 2005;14: 2840-2847.
7. Birgegård G, Aapro MS, Bokemeyer C, Dicato M, Drings P, Hornedo J, et al. Cancer-related anemia: pathogenesis, prevalence and treatment. Oncology. 2005; 68: 3-11.
8. Nie D, Yang E, Li Z. Pretreatment thrombocytosis predict poor prognosis in patients with endometrial carcinoma: a systematic review and meta-analysis. BMC Cancer. 2019; 19: 73.
9. Ye Q, Wu Z, Xia T, Liu D, Yang Y, et al. Pre-treatment thrombocytosis predicts prognosis of endometrial cancer: A meta-analysis of 11 studies. Exp Ther Med. 2020; 19: 359-366.
10. Worley MJ, Nitschmann CC, Shoni M, Vitonis AF, Rauh-Hain JA, et al. The significance of preoperative leukocytosis in endometrial carcinoma. Gynecol Oncol. 2012; 125: 561-565.
11. Njolstad TS, Engerud H, Werner HM, Salvesen HB, Trovik J. Preoperative anemia, leukocytosis and thrombocytosis identify aggressive endometrial carcinomas. Gynecol Oncol. 2013; 131: 410-415.
12. Tamussino KF, Gucer F, Reich O, Moser F, Petru E, et al. Pretreatment hemoglobin, platelet count, and prognosis in endometrial carcinoma. Int J Gynecol Cancer. 2001; 11: 236-240.
13. Bai YY, Du L, Jing L, Tian T, Liang X, Jiao M, et al. Clinicopathological and prognostic significance of pretreatment thrombocytosis in patients with endometrial cancer: a meta-analysis. Cancer Manag Res. 2019; 11: 4283-4295.
14. Salem H, Abu-Zaid A, Aloman O, Abuzaid M, Alsabban M, et al. Preoperative Leukocytosis as a Prognostic Marker in Endometrioid-Type Endometrial Cancer: A Single-Center Experience from Saudi Arabia. Gulf J Oncolog. 2020; 1: 51-58.
15. Abu-Zaid A, Alomar O, Baradwan S, Abuzaid M, Alshahrani MS, et al. Preoperative leukocytosis correlates with unfavorable pathological and survival outcomes in endometrial carcinoma: A systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2021; 264: 88-96.
16. Abu-Zaid A, Alomar O, Abuzaid M, Baradwan S, Salem H, Al-Badawi IA. Preoperative anemia predicts poor prognosis in patients with endometrial cancer: A systematic review and metaanalysis. Eur J Obstet Gynecol Reprod Biol. 2021; 258: 382-390.

17. Nordsmark M, Overgaard J. Tumor hypoxia is independent of hemoglobin and prognostic for loco-regional tumor control after primary radiotherapy in advanced head and neck cancer. Acta Oncol. 2004; 43: 396-403.

18. Durand RE. Keynote address: the influence of microenvironmental factors on the activity of radiation and drugs. Int J Radiat Oncol Biol Phys. 1991; 20: 253-258.

19. Moon EJ, Petersson K, Olcina MM. The importance of hypoxia in radiotherapy for the immune response, metastatic potential and FLASH-RT. Int J Radiat Biol. 2022; 98: 439-451.

20. Visser NC, Bulten J, van der Wurff AA, Boss EA, Bronkhorst CM, Feijen HW, et al. PIpelle Prospective ENDOmetrial carcinoma (PIPENDO) study, pre-operative recognition of high risk endometrial carcinoma: a multicentre prospective cohort study. BMC Cancer. 2015; 15: 487.

21. Bouwman F, Smits A, Lopes A, Das N, Pollard A, et al. The impact of BMI on surgical complications and outcomes in endometrial cancer surgery--an institutional study and systematic review of the literature. Gynecol Oncol. 2015; 139: 369-376.

22. Gynaecologie WO. Landelijke richtlijn endometriumcarcinoom 2018.

23. Sundar S BJ, Crosbie E, Drake A, Edmondson R, Fotopoulou C, et al. BGCS Uterine Cancer Guidelines: Recommendations for Practice 2017 [23-6-2021].

24. McLean E, Cogswell M, Egli I, Wojdyla D, de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr. 2009; 12: 444-454.

25. Moliterno AR, Spivak JL. Anemia of cancer. Hematol Oncol Clin North Am. 1996; 10: 345-363.

26. Obermair A, Handisurya A, Kaider A, Sevelda P, Kolbl H, et al. The relationship of pretreatment serum hemoglobin level to the survival of epithelial ovarian carcinoma patients: a prospective review. Cancer. 1998; 83: 726-731.

27. Wilairat W, Benjapibal M. Presence of anemia and poor prognostic factors in patients with endometrial carcinoma. Asian Pac J Cancer Prev. 2012; 13: 3187-3190.

28. Berridge MV, Fraser JK, Carter JM, Lin FK. Effects of recombinant human erythropoietin on megakaryocytes and on platelet production in the rat. Blood. 1988; 72: 970-977.

29. Li N. Platelets in cancer metastasis: To help the «villain» to do evil. Int J Cancer. 2016; 138: 2078-2087.

30. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454: 436-444.