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Nuno Rafael Vieira Cardoso

A amplitude de distribuição dos glóbulos vermelhos como preditor de eventos na doença aorto-ilíaca extensa

Red cell distribution width as a predictor of cardiovascular outcomes in extensive aortoiliac disease

FMUP

MARÇO, 2022

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UC Dissertação/Projeto (6º Ano) - DECLARAÇÃO DE INTEGRIDADE

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Red blood cell distribution width as a predictor of cardiovascular outcomes in extensive aortoiliac disease

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Faculdade de Medicina da Universidade do Porto, 21/03/2022

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Para os meus avós

Sísifo

Recomeça…

Se puderes,

Sem angústia e sem pressa.

E os passos que deres,

Nesse caminho duro

Do futuro,

Dá-os em liberdade.

Enquanto não alcances

Não descanses.

De nenhum fruto queiras só metade.

E, nunca saciado,

Vai colhendo

Ilusões sucessivas no pomar.

Sempre a sonhar

E vendo,

Acordado,

O logro da aventura.

És homem, não te esqueças!

Só é tua a loucura

Onde, com lucidez, te reconheças.

Miguel Torga [1977], Diário: Vols. XIII a XVI. Lisboa: D. Quixote, [s.d.], p. 20.

Red blood cell distribution width as a predictor of cardiovascular outcomes in extensive aortoiliac disease

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Running title: Red blood cell distribution width and aortoiliac disease

Abstract

Background: Aortoiliac peripheral artery disease may lead to disabling lower limb claudication or to lower limb chronic threatening ischemia, which is associated with increased short and long-term morbi-mortality. The red blood cell distribution width-coefficient of variation (RDW-CV) has been able to predict outcomes in other atherosclerotic diseases, such as myocardial infarction and stroke. The main objective of this study was to assess the predictive ability of perioperative RDW-CV in accurately predicting short and long-term major adverse cardiovascular events (MACE) and all-cause mortality in patients submitted to aortoiliac revascularization due to extensive aortoiliac atherosclerotic disease.

Methods: From 2013 to 2020, patients who underwent aortoiliac revascularization due to severe aortoiliac disease were included in a prospective cohort. Blood samples were taken preoperatively and the patient's demographics, comorbidities, and postoperative outcomes were assessed. A multivariate Cox regression model was used to adjust for confounding and assess the independent effect of these prognostic factors on the outcomes.

Results: The study group included 107 patients. Median follow-up was 57 (95% CI 34.4-69.6) months. Preoperative RDW-CV was increased in thirty-eight patients (35.5%). Increased RDW-CV was associated with congestive heart failure - adjusted odds ratio of 5.043 (95% CI 1.436-17.717, p=0.012). It could predict long-term occurrence of MACE (adjusted hazard ratio, aHR=1.065, 95% CI 1.014-1.118, p=0.011), all-cause mortality (aHR=1.069, 95% CI 1.014-1.126, p=0.013), acute heart failure (AHF) (aHR=1.569, 95% CI 1.179-2.088, p=0.002), and stroke (aHR=1.343, 95% CI 1.044-1.727, p=0.022).

Conclusions: RDW-CV is a widely available and low-cost marker that was able to independently predict long-term AHF, stroke, MACE, and all-cause mortality in patients with extensive aortoiliac disease submitted to revascularization. This biomarker could help assess which patients would likely benefit from stricter follow-up in the long-term.

Introduction

Aortoiliac peripheral artery disease is defined by atherosclerosis that affects the infrarenal aorta and the iliac arteries. Aortoiliac disease typically occurs in younger individuals and is more rapidly progressive than distal artery disease (1). In advanced stages, it is responsible for disabling lower limb claudication or even lower limb chronic threatening ischemia contributing to a decrease in quality of life and increased morbi-mortality for these patients (2).

The definitive treatment for severe aortoiliac disease remains unclear although until recently the gold standard used to be open surgery mainly through aortobifemoral bypass grafting (3, 4). Besides open surgery, endovascular can also be offered, each one carrying its advantages. The endovascular approach is significantly less invasive than open surgery, providing lower complications rates, shortening hospitalization times, and decreasing hospital costs (5), with short-term patency and limb results comparable to the open surgery approach (6). On the other hand, the open surgery approach has a higher technical success rate and is not limited by a previous endovascular approach. Therefore, an "endovascular-first" approach is increasingly being offered in severe aortoiliac disease (6). A recent meta-analysis including 9319 patients revealed a better 30-day morbi-mortality for endovascular techniques, yet open surgery maintained better primary patency in both early and midterms. Nonetheless, secondary patency was comparable (7).

Hematological parameters, such as the red blood cell distribution width (RDW), have recently been emerging as a tool to help predict patient outcomes. The RDW is a parameter that reveals erythrocyte size distribution and is present in a routine complete blood cell count (CBC). Automated hematological cell counters calculate RDW as a coefficient of variation (CV), and the final result is expressed as a percentage, generally encompassing 12% to 15% (8).

Increased RDW-CV was initially acknowledged as a marker of iron deficiency. However, several additional potential mechanisms are suggested as the cause of increased levels of RDW-CV, such as nutritional deficiency (vitamin B12, folic acid), bone marrow depression, or inflammation, which can lead to the prolongation of red blood cell lifespan (9). RDW-CV has been extensively studied among cardiovascular patients and has been associated with increased morbidity and mortality in this setting (10-12) and independently predict mortality in coronary artery disease (CAD) and ischemic stroke (13, 14). Additionally, RDW-CV has also demonstrated its prognostic value in other diseases, such as pancreatic (15), end-stage renal (16), liver diseases (17), sepsis (18), and even malignancies (19, 20).

Considering the prognostic ability of RDW-CV, one could conjecture its relevance in predicting postoperative and long-term outcomes after aortoiliac revascularization and, therefore, help to identify patients at higher risk of adverse events. This study aimed to evaluate the potential role of perioperative hematologic parameters, such as RDW-CV, in predicting long-term major adverse cardiovascular events (MACE) and all-cause mortality. As secondary outcomes, this work aimed to determine the prognostic value of RDW-CV by testing its association with the previously mentioned long-term adverse outcomes, including acute myocardial infarction (AMI), stroke, acute heart failure (AHF), and major adverse lower limb events (MALE).

Methods

Patient Selection

Between January 2013 and January 2021, consecutive patients who underwent elective aortoiliac revascularization were included in this prospective cohort study. All patients were selected from a tertiary and a community hospital and had aortoiliac TransAtlantic Inter-Society Consensus (TASC) II type D lesions, excluding those with aortoiliac aneurysmatic disease (3). The choice between open surgery or an endovascular procedure was made by a shared decision between the patient and the surgeon, taking into account the surgeon and the institution's experience and preferences.

The demographic and clinical characteristics of patients, including their cardiovascular risk factors and their procedural and lesion-specific details, were retrieved by a detailed review of their clinical records, including computed tomography scans (6). Further details regarding the type of lesion are described elsewhere (3). Patients were assessed in the first 30-days post-procedure and in the subsequent long-term surveillance period. Reported outcomes included patient-related events such as AHF, AMI, stroke, and all-cause mortality. Additionally, limb-related events such as reintervention, acute limb ischemia, or occlusion without intervention were also accounted for. This study is under the framework of the Strengthening the Reporting of Cohort Studies in Surgery (STROCSS) 2019 Guideline (21).

The study protocol was approved by the local Ethics Committee and respects the Declaration of Helsinki. Patient informed consent was handled accordingly, and all data processing was anonymous (Protocol Number 246-18).

Definitions

Retrieved data was registered in agreement with the Reporting Standards of the Society for Vascular Surgery for lower extremity ischemia (22). The Rutherford chronic ischemia classification was used to classify the symptoms and severity of chronic lower extremity ischemia (23).

The technical success of the operation was defined as maintenance of patency 24 hours after the procedure. MACE was defined as a composite outcome, including AMI, AHF and all-cause mortality (24). Major adverse limb events (MALE) were defined as the combined events of reintervention, including reintervention due to primary assisted patency, secondary patency or major amputation of the revascularized artery segment, and occlusion without intervention (25).

The Δ RDW-CV was defined as the difference between postoperative (24h) and preoperative RDW-CV levels.

Analytical parameters

All patients had at least one sample of preoperative CBC available in the two weeks preceding the intervention. When several blood samples per patient met inclusion criteria (<2 weeks before surgery/intervention), the sample of choice for analysis was the closest sample before surgery. Blood analysis was executed using a Sysmex® XE-2100D automated hematology analyzer, with reference values for RDW-CV within 11% and 16%.

Statistical Analysis

The sample needed for a survival test was calculated applying WinPepi® V11.65, aiming for a statistical power (β) of 90% and an $\alpha < 0.05$. The sample was estimated (95 patients) for a hazard ratio of 2 between groups and a predicted survival at the end of follow-up of 80%, although higher hazards are described (6, 26).

For statistical analysis, SPSS (IBM Corp., released 2019. IBM SPSS Statistics for Windows, version 27.0, Armonk, NY, USA) was used. Student's t-test was favored when dealing with normally distributed continuous variables, while the Mann-Whitney-U was privileged when working with variables whose normal distribution could not be assumed, presenting either as mean and standard deviation or median and range, respectively. For the analysis of categorical variables, the Chi-squared test was used. Statistically significant results were set at a $p < 0.05$ level.

To ease the understanding and clinical use of the RDW-CV, the authors opted to establish a threshold. The best cut-off for RDW was obtained, resorting to an optimal binning.

Multivariate logistic regression analysis was performed to determine independent clinical and demographic factors that were associated with RDW-CV. The backward stepwise regression method was applied, and variables with $p < 0.10$ were included. The Log Rank estimator was utilized to test the effect of the RDW-CV and Δ RDW-CV in time-dependent variables. Multivariate Cox regression analysis was performed for independent predictors of long-term MACE and all-cause mortality according to the backward stepwise regression method.

Results

Population Data

The cohort included a total of 107 participants, 95.3% male, with a median age of 62.2 ± 8.84 years. Thirty-eight patients had increased preoperative RDW-CV (35.5%). There were no significant differences in age or gender between the RDW-CV groups. The median follow-up of the cohort was 57 (95% CI 34.4-69.6) months.

Regarding comorbidities, there was a significant association between elevated RDW-CV and both CAD (21.7% vs 42.1%, p=0.026) and congestive heart failure (CHF: 5.8% vs 23.7%, p=0.007). No association was found between the remaining comorbidities evaluated. (Table I)

Concerning hematologic parameters, an elevated RDW-CV was significantly associated with decreased preoperative hemoglobin (mean 13.42±1.76 vs. 12.44±2.13 g/dl, p=0.012) and increased preoperative neutrophils count (5.51±2.42 vs. 6.09±3.20 *10^9L, p=0.049). (Table II)

Multivariate logistic regression analysis

After multivariate analysis, the only comorbidity for which the association with a higher RDW-CV remained statistically significant was CHF, with an adjusted odds ratio (aOR) of 5.043 (95% CI 1.436-17.717, p=0.012). (Table I)

Multivariate analysis did not confirm a significant independent association between increased RDW-CV and preoperative hemoglobin or neutrophils. (Table II)

Predictive Ability of RDW-CV on Short-Term Outcomes

Regarding short-term outcomes, there was no significant association between increased RDW-CV and the 30-day incidence of MACE (9.9% vs 10.5%, p=0.912), prosthetic infection (3% vs 2.7%, p=0.934). The difference (Δ) between preoperative and postoperative ABI (Δ 0.46±0.22 vs 0.38±0.25, p=0.236) or the Rutherford chronic ischemia classification (Δ 2.55±1.44 vs 2.46±1.56, p=0.819) were not significant between groups. (Table III)

Predictive Ability of RDW-CV on Long-Term Outcomes

Log-rank univariate analysis of RDW-CV showed a statistically significant association between increased preoperative RDW-CV and long-term occurrence of MACE (p=0.041). No statistically significant association was found between increased preoperative RDW-CV and long-term AMI, AHF, stroke or MALE. (Figure 1)

RDW and other prognostic factors were included in the multivariate Cox regression model to adjust for confounding and assess the independent effect of these factors on the primary and secondary outcomes. Cox multivariate regression proportional hazard ratio revealed a statistically significant adjusted hazard ratio (aHR) between increased RDW-CV and AHF (aHR=1.569, 95% CI 1.179-2.088, p=0.002), stroke (aHR=1.343, 95% CI 1.044-1.727, p=0.022), MACE (aHR=1.065, 95% CI 1.014-1.118, p=0.011) and all-cause mortality (aHR=1.069, 95% CI 1.014-1.126, p=0.013). Additionally, it also revealed that an increased Δ RDW-CV had a statistically significant relation with MACE (aHR=1.492, 95% CI 1.096-2.033, p=0.011) and all-cause mortality (aHR=1.516, 95% CI 1.050-2.189, p=0.026). (Table IV)

Cox proportional hazard multivariable analysis of prognostic factors showed a statistically significant aHR between MACE and RDW-CV (aHR=1.209, 95% CI 1.043-1.402, p=0.012), Δ RDW-CV (aHR=1.867, 95% CI 1.337-2.606, p=0.001), chronic kidney disease (CKD; aHR=2.895, 95% CI 1.311-6.392, p=0.09) and CHF (aHR=3.636, 95% CI 1.508-8.763, p=0.04), as well as between all-cause mortality and RWD-CV (aHR=1.253, 95% CI 1.065-1.475, p=0.007), Δ RDW-CV (aHR=2.019, 95% CI 1.348-3.025, p=0.001), CKD (aHR=2.949, 95% CI 1.215-7.158, p=0.017) and CHF (aHR=4.429, 95% CI 1.698-11.557, p=0.02). (Figure 2)

Discussion

This study found that an elevated preoperative RDW-CV can predict long-term outcomes such as AHF, stroke, MACE, and all-cause mortality in patients undergoing revascularization due to extensive aortoiliac disease. Additionally, Δ RDW-CV was an independent predictor of long-term all-cause mortality and MACE. No association was found regarding short-term outcomes.

As mentioned above, RDW-CV has been thoroughly studied not only in the setting of cardiovascular diseases but in various other pathologies. The mechanisms by which RDW-CV is associated with worse prognosis, including mortality, remain unclear. Its pathophysiology might be explained by a combination of several mechanisms, including decreased erythrocyte deformability (27), anemia, increased inflammatory cytokines, and oxidative stress (28, 29). An explanation for the relationship between increased RDW-CV and mortality might be the occurrence of chronic low-grade inflammation. Lippi et al., studying a retrospective cohort of 3845 patients, found that high levels of RDW-CV were associated with increased inflammatory markers (30).

These markers activate platelets and the endothelium, promoting atherogenesis (31) and may subsequently increase mortality (32).

Our findings regarding increased RDW-CV and its ability to predict the long-term occurrence of stroke, AHF, MACE, and all-cause mortality in patients submitted revascularization due to advanced aortoiliac disease are consistent with previous studies, albeit these studies were conducted in different patient populations such as those with atrial fibrillation, coronary artery disease and carotid artery disease (26, 33, 34).

The variation between RDW-CV at the time of admission and subsequent postoperative RDW-CV (Δ RDW-CV) has been documented as a short and mid-term predictor of mortality in patients with acute heart failure (35, 36). Lee et al. reported that a higher Δ RDW in patients undergoing coronary artery bypass grafting was able to predict short-term adverse outcomes (37). Veraldi et al. found that the ratio between preoperative and postoperative RDW-CV values could predict short-term procedure-related complications in patients subjected to percutaneous transluminal angioplasty (38). Regarding the abdominal aorta, Veraldi et al. also reported that an increased RDW-CV ratio was significantly associated with the occurrence of post-implantation syndrome after endovascular repair in patients with abdominal aortic aneurysm (39). In our study, Δ RDW-CV was calculated to assess the impact of the procedure on RDW-CV levels, and we found that increased Δ RDW-CV could predict all-cause mortality and MACE in patients undergoing aortoiliac revascularization. This finding may aid in selecting patients who require a tighter follow-up, especially patients with increased values of preoperative RDW-CV.

Concerning demographic comorbidities, in our study, RDW-CV was statistically and independently associated with CHF (Table I). The pathophysiology of this relationship is unclear. It is known that CHF is associated with a proinflammatory state,

with the production of cytokines and acute-phase reactants, such as tumor necrosis α, interleukin 1α, interleukin 6, and interferon α (40) and that RDW-CV may be associated with these inflammatory cytokines (41). Furthermore, anemia has been extensively studied as a strong prognostic factor for CHF (42, 43), and it can lead to elevations in RDW-CV. Recently, some studies have shown that iron supplementation in both anemic and non-anemic CHF patients has improved functional status and quality of life in patients, which suggests that iron metabolism is a crucial element of the pathophysiology of CHF (44). Iron deficiency, defined by an increased RDW with a decreased mean corpuscular volume (MCV), carries a worse prognosis than isolated increased RDW-CV or decreased hemoglobin (45). However, some studies reported that RDW-CV remained an independent predictor of outcome after adjusting for iron deficiency (46), indicating that the underlying problem may be impaired iron mobilization with normal total body iron rather than iron deficiency. RDW seems to be a prominent marker of anemia of chronic diseases complicated with iron deficiency in patients with HF (47). The association between elevated RDW-CV and low hemoglobin, reduced MCV, normal iron-binding capacity, and normal ferritin are consistent with a state of impaired iron mobilization (48).

In the present study, CKD was found to be an independent predictor of mortality and MACE. In the literature, increased RDW-CV has been associated with cardiovascular adverse events and mortality in CKD patients (49, 50). However, in this study, a statistical association between the prevalence of CKD and the preoperative RDW-CV could not be found. This issue should be further studied with the inclusion of more patients.

Limitations

There are some limitations inherent to this study that need consideration. First, the possibility of the occurrence of a selection bias is not negligible, seen as patients with

more comorbidities and with an unfavorable condition may have been selected for less invasive procedures or even ruled out from revascularization. The current recommended guidelines for extensive aortoiliac disease also consider open surgery as the first-line treatment on healthy patients and that an endovascular-first approach should be considered in patients with more severe comorbidities (4). Second, this is a two-center study with a relatively small number of patients, and males were overrepresented, limiting its generalization to the female population undergoing aortoiliac revascularization, although they consist of a minority of these patients. Absolute values of RDW and its reference intervals are influenced by several factors including not only gender but also age or ethnicity (51, 52).

On the other hand, the prospective nature of the design, the extended follow-up, and the similarity between the aortoiliac lesions of the patients are some of the strengths of our study. This work includes patients from a large academic teaching institution and from a community referral hospital, which increases the external validity of the results. Nonetheless, further studies are necessary to confirm these findings.

Conclusions

RDW-CV is a ubiquitous, low-cost, easy-to-obtain hematological marker that can independently predict long-term mortality, MACE, stroke and AHF after aortoiliac revascularization due to extensive disease. The integration of RDW-CV and Δ RDW-CV into prognostic scores may help identify patients with an increased risk of developing worse outcomes that would benefit from an intensive follow-up protocol.

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Notes

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Table I - Patient's demographics and comorbidities

*Characteristics**	Total n = 107	RDW CV≤14.1 (n=69)	RDW CV >14.1 (n=38)	P=	Multivariable Analysis CI 5-95%
Age, years	62.2 ± 8.84	62.3±9.02	61.8±10.7	0.444	
Sex, male	102 (95.3)	66 (94.3)	46 (94.7)	0.922	
Hypertension	70 (65.4)	48 (69.6)	22 (57.9)	0.225	
Smoking history	96 (89.7)	64 (91.4)	32 (84.2)	0.254	
Diabetes	34 (31.7)	22 (32.4)	12 (31.6)	0.935	
Dyslipidemia	67 (62.6)	43 (62.3)	24 (63.2)	0.932	
CKD	16 (15)	8 (11.6)	8 (21.1)	0,189	
CAD	31 (29)	15 (21.7)	16 (42.1)	0.026	NC
COPD	10 (9.3)	4 (5.8)	6 (15.8)	0.089	NC
CHF	13 (12.1)	4 (5.8)	9 (23.7)	0.007	**5.043 (1.436-17.717)** **P=0.012**
ASA *2* *3* *4*	 38 (35.5) 62 (57.9) 7 (6.5)	 26 (37.7) 40 (58) 3 (4.3)	 12 (31.6) 22 (57.9) 4 (10.5)	0.430	
SFA disease, n (%)	65 (61.9)	41 (61.2)	24 (63.2)	0.842	
Rutherford Classification *III* *IV*	 28 (26.1) 46 (43)	 17 (24.6) 30 (43.5)	 11 (28.9) 16 (42.1)	0.765	

V *VI*	27 (25.2) 6 (5.6)	19 (27.5) 3 (4.3)	8 (21.1) 3 (7.9)		
Gangrene	13 (13.8)	7 (10.9)	6 (20)	0.235	
Iliac Stenting (vs ABF) *n (%)*	40 (37)	27 (38.6)	13 (34.2)	0.654	
MFI5					
ABI	0.3 ± 0.12	0.3 ± 0.12	0.3 ± 0.14	0.996	

*Variables are presented as N (%) if categorical, mean±standard deviation if continuous and median (interquartile range if ASA).

ABF – aortobifemoral bypass; ASA - American Society of Anesthesiologists; CAD - Coronary artery disease; CHF – Congestive heart failure; CKD - Chronic kidney disease (creatinine = 1.5 mg/dl); COPD - Chronic obstructive pulmonary disease; Pre-op Hb - pre-operative hemoglobin; PAD - Peripheral artery disease; mFI-5 - Modified Frailty index – 5; RDW CV – red cell distribution width coefficient of variation.

Table II - Hematologic parameters

	Study population			
Preoperative	RDW CV≤14.1 (n=69)	RDW CV >14.1 (n=38)	P-Value	Multivariable aOR CI 5-95
Hemoglobin (g/dl)	13.42±1.76	12.44±2.13	0.012	NC
RDW-CV (%)	13.08±0.536	15.45±2.00	0.001	NI
RDW-SD (fL)	44.08±2.96	51.11±5.83	0.001	NI
WBC	9.067±2.65	9.123±3.43	0.925	
Lymphocyte count	2.49±1.32	2.11±0.81	0.112	
Lymphocyte (%)	28.6±12.1	24.1±9.6	0.053	NC
Neutrophil count	5.51±2.42	6.09±3.20	0.049	NC
Neutrophil (%)	59.5±12.3	64.3±11.4	0.289	
Platelet count	26642±91.84	24755±76,82	0.950	
MPV (fL)	10.74±0.984	10.40±0.721	0.400	
PDW (fL)	12.88±1.873	11.98±1.362	0.880	
Eosinophiles Count	0.256±0.315	0.253±0.319	0.953	
Basophiles Count	0.0311±0.03258	0.0642±0.14726	0.179	

Monocytes	0.9717±1.289	1.2797±1.789	0.303	
Neutrophil / Lymphocyte ratio	2.66±1.69	3.30±2.10	0.087	
Platelet / Lymphocyte ratio	12453±64.64	12915±53.766	0.708	
Hemoglobin /Platelet ratio	0.057±0.0223	0.0563±0.0236	0.937	
Postoperative				
Hemoglobin (g/dl)	10.4±1.53	9.9±1.50	0.186	
RDW-CV (%)	13.7±1.13	16.0±2.08	**0.001**	NI
RDW-SD (fL)	46.1±4.6	52.8±6.30	0.001	NI
WBC	46.2±4.56	52.8±6.30	0.349	
Lymphocyte count	1.6±0.95	1.44±0.58	0.338	
Lymphocyte (%)	14.2±7.39	14.2±8.4	0.993	
Neutrophil count	9.0±3.35	10.8±10.2	0.216	
Neutrophil (%)	75.7±9.44	76.37±10.48	0.752	
Platelet count	20448±78.9	20347±74,941	0.950	
MPV (fL)	10.83±0.834	10.43±0.955	0.400	

PDW (fL)	12.63±1.91	12.53±0.423	0.880	
Eosinophiles Count	0.9±1.91	0.7±0.92	0.597	
Basophiles Count	0.15±0.106	0.19±0.180	0.299	
Monocytes	8.7±3.02	8.2±3.19	0.445	
Neutrophil / Lymphocyte ratio	7.52±5,426	8.13±6.83	0.627	
Platelet / Lymphocyte ratio	0.75±0.419	0.71±0.404	0.769	
Hemoglobin /Platelet ratio	1.35±0.527	1.58±1.359	0.783	
RDW CV Delta (%)	0.57±0.865	0.51±1.13	0.743	

Legend: aOR CI 5-95 – adjusted Odds ratio confidence interval 5-95; MCHC – mean corpuscular hemoglobin concentration; MPV – mean platelet volume; NC – not confirmed on multivariable analysis; NI – Not included in the multivariable analysis; PDW – Platelet distribution width; RDW-CV — red blood cell distribution width coefficient of variation; RDW-SD — red blood cell distribution width standard deviation.

Table III – Patient's 30 day outcomes according to RDW-CV score

	MACE n (%)	P=	Prosthetic infection n (%)*	P=	ABI Δ	P=	Rutherford Δ	P=
RDW-CV ≤14.1%	7 (9.9)	0.912	2 (3)	0.934	0.46 ± 0.22	0.236	2.55±1.44	0.810
RDW-CV >14.1%	4 (10.5)		1 (2.7)		0.38 ± 0.25		2.46±1.56	

Legend: ABI – Ankle brachial index Δ - preoperative minus postoperative; Rutherford chronic ischemia Δ – preoperative minus postoperative; MACE – Major Adverse Cardiovascular Event at 30 Days; Prothesis infection (*1 Year of follow-up); RDW-CV — red blood cell distribution width coefficient of variation;

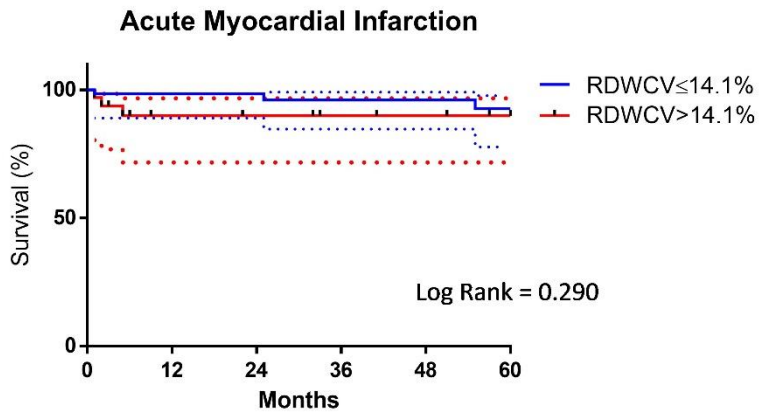
Table IV - Cox multivariate regression proportional hazard ratio for each outcome

	Non-adjusted Hazard Ratios	95% Confidence interval	P-Value
AMI			
RDW CV	1.007	0.891-1.139	0.906
RDW CV Δ	1.209	0.631-2.315	0.568
AHF			
RDW CV	**1.569**	**1.179-2.088**	**0.002**
RDW CV Δ	0.852	0.154-4.707	0.854
Stroke			
RDW CV	**1.343**	**1.044-1.727**	**0.022**
RDW CV Δ	1.210	0.450-3.251	0.705
MALE			
RDW CV	0.654	0.707-1.243	0.654
RDW CV Δ	1.426	0.991-2.053	0.056
MACE			
RDW CV	**1.065**	**1.014-1.118**	**0.011**
RDW CV Δ	**1.492**	**1.096-2.033**	**0.011**
All-cause mortality			
RDW CV	**1.069**	**1.014-1.126**	**0.013**
RDW CV Δ	**1.516**	**1.050-2.189**	**0.026**

AHF – Acute Heart Failure; AMI – Acute Myocardial Infarction; MALE - Major Adverse Limb Events; MACE – Major Adverse Cardiovascular Events; RDW-SD — red blood cell distribution width – coefficient of variation

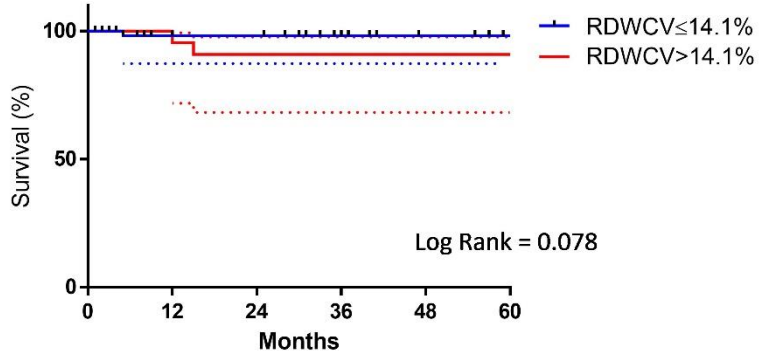
Fig.1 - Survival plots with log-rank analysis. Sixty-month follow-up Kaplan-Meyer curves for different outcomes following aortoiliac revascularization for groups with or without increased RDW-CV. Freedom from AMI (a), AHF (b), stroke (c), MALE (d), MACE (e), and all-cause mortality (f) after aortoiliac revascularization according to RDW-CV. Survival tables display the percent freedom from an event (1st row), standard error (SE; 2nd row), number of events (3rd row), and number of subjects free from an event (4th row).

a



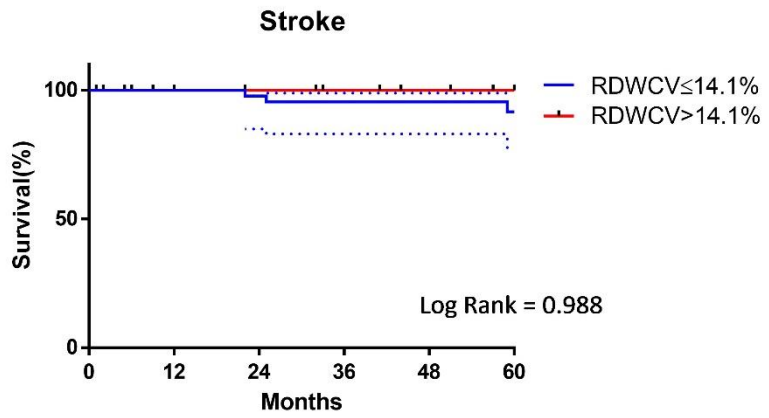
Variable		30d	12	24	36	48	60
RDW CV≤14.1%	Suvival(%)	97.2%	97.2%	94.9%	94.9%	91.6%	91.6%
	SE (%)	2.0%	2.0%	2.9%	2.9%	4.3%	4.3%
	n	61	44	42	32	28	24
		2	2	3	3	4	4
RDW CV>14.1%	Suvival(%)	92.1%	85.7%	85.7%	85.7%	85.7%	85.7%
	SE (%)	4.4%	6.0%	6.0%	6.0%	6.0%	6.0%
	n	33	20	19	16	15	12
		3	5	5	5	5	5

b



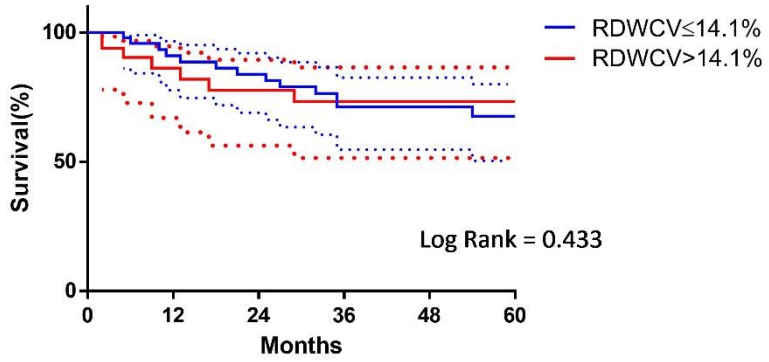
Variable		30d	12	24	36	48	60
RDW CV≤14.1%	Suvival(%)	100%	98.2%	98.2%	98.2%	98.2%	98.2%
	SE (%)	0.0%	1.8%	1.8%	1.8%	1.8%	1.8%
	n	63	44	44	33	28	24
		0	1	1	1	1	1
RDW CV>14.1%	Suvival(%)	97.4%	92.9%	88.5%	88.5%	88.5%	88.5%
	SE (%)	2.6%	5.0%	6.4%	6.4%	6.4%	6.4%
	n	35	21	20	19	15	12
		1	2	3	3	3	3

c



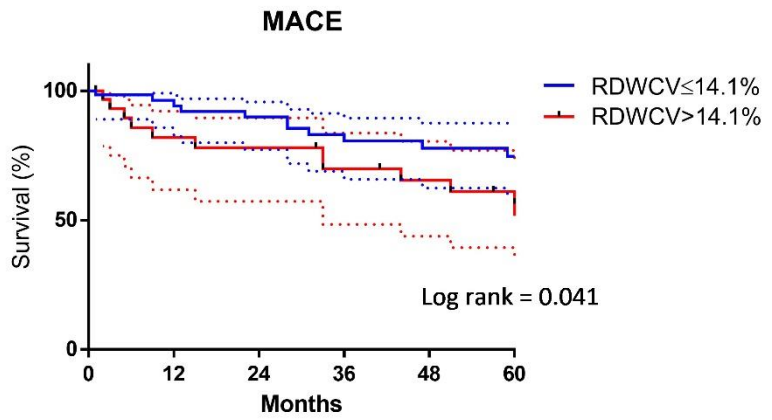
Variable		30d	12	24	36	48	60
RDW CV≤14.1%	Suvival(%)	100%	100%	95.6%	95.6%	95.6%	91.9%
	SE (%)	0.0%	0.0%	3.1%	3.1%	3.1%	4.7%
	n	63	45	43	33	28	24
		0	0	2	2	2	3
RDW CV>14.1%	Suvival(%)	94.7%	94.7%	94.7%	94.7%	94.7%	94.7%
	SE (%)	3.6%	3.6%	3.6%	3.6%	3.6%	3.6%
	n	34	21	20	17	15	12
		2	2	2	2	2	2

d



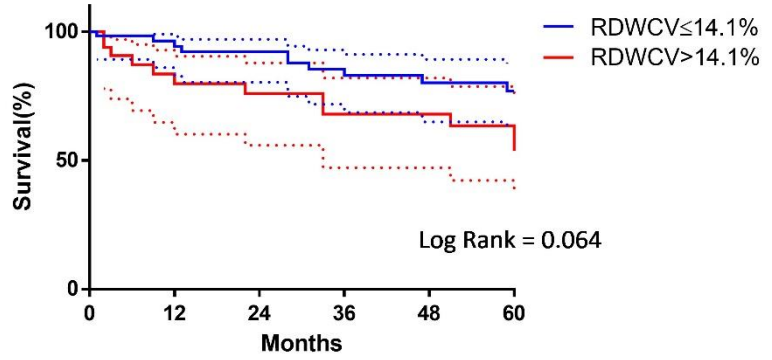
Variable		30d	12	24	36	48	60
RDW CV≤14.1%	Suvival(%)	88.7%	80.7%	74.4%	63.1%	63.1%	60.0%
	SE (%)	3.8%	5.1%	5.9%	6.8%	6.8%	7.2%
	n	56	38	35	26	20	15
		8	12	15	20	20	21
RDW CV>14.1%	Suvival(%)	97.4%	84.0%	75.6%	71.4%	71.4%	71.4%
	SE (%)	2.6%	6.7%	8.3%	8.8%	8.8%	8.8%
	n	35	20	18	16	14	12
		1	5	7	8	8	8

e



Variable		30d	12	24	36	48	60
RDW CV≤14.1%	Suvival(%)	93%	87.5%	83.5%	74.9%	72.3%	69.4%
	SE (%)	3%	4.2%	4.9%	6%	6.3%	6.7%
	n	61	44	42	32	28	24
		5	8	10	14	15	16
RDW CV>14.1%	Suvival(%)	89.5%	73.3%	69.8%	62.4%	58.5%	50.4%
	SE (%)	5.0%	7.7%	8.0%	8.8%	9.1%	9.4%
	n	32	21	20	17	15	12
		4	9	10	12	13	15

f



Variable		30d	12	24	36	48	60
RDW CV≤14.1%	Suvival(%)	95.8%	90.3%	88.3%	79.5%	76.7%	73.7%
	SE (%)	2.4%	3.8%	4.2%	5.7%	6.1%	6.6%
	n	63	45	44	33	28	24
		3	6	7	11	12	13
RDW CV>14.1%	Suvival(%)	97.4%	77.7%	74.0%	66.2%	66.2%	57.0%
	SE (%)	2.6%	7.5%	8.0%	8.9%	8.9%	9.7%
	n	36	21	20	17	15	12
		1	7	8	10	10	12

Legend: AHF - acute heart failure; AMI - acute myocardial infarction; MACE - major adverse cardiovascular events; MALE - major adverse limb events; RDW-CV - red cell distribution width-coefficient of variation;

Fig.2 – Cox proportional hazard multivariable analysis of prognostic factors for MACE (2.1) and All-cause Mortality (2.2)

Fig.2.1

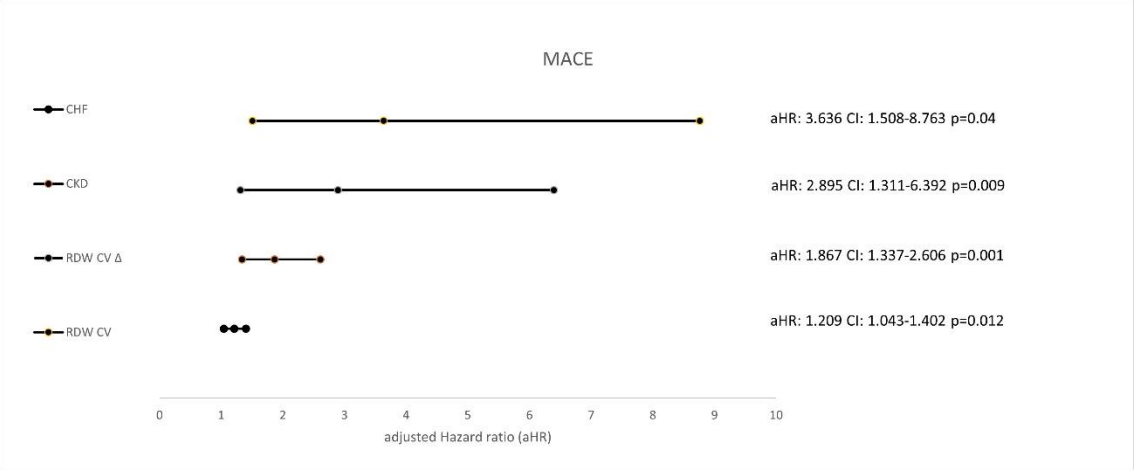
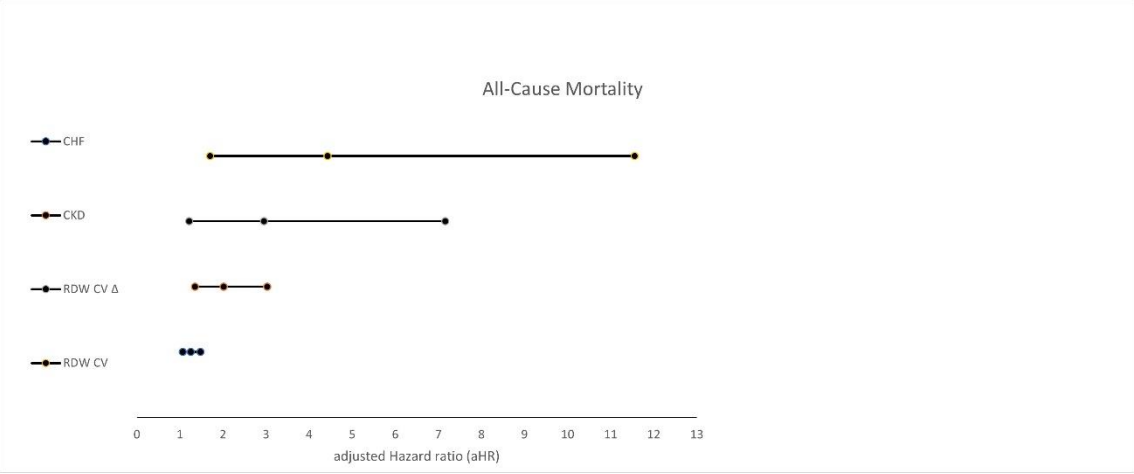


Fig.2.2



Legend: aHR – adjusted Hazard ratio; CHF – Congestive Heart Failure; CKD - Chronic kidney disease; MACE - Major Adverse Cardiovascular Events; RDW-CV — red blood cell distribution width – coefficient of variation; Δ RDW-CV – red blood cell distribution width – coefficient of variation delta.

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Page 3 (Paragraph 2): "From 2013 to 2020, patients who underwent aortoiliac revascularization due to severe aortoiliac disease were included in a prospective cohort."
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Page 3 (Paragraph 2): "From 2013 to 2020, patients who underwent (…) acute heart failure (AHF) (aHR=1.569, 95% CI 1.179-2.088, p=0.002), and stroke (aHR=1.343, 95% CI 1.044-1.727, p=0.022)."
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Pages 5-6: "Hematological parameters, such as the red blood cell distribution width (RDW), (…) help to identify patients at higher risk of adverse events."
Objectives	3	State specific objectives, including any prespecified hypotheses	Page 6 (Paragraph 2): "This study aimed to evaluate the potential role (…) and major adverse lower limb events (MALE)."
Methods			
Study design	4	Present key elements of study design early in the paper	Page 7 (Paragraph 1): "Between January 2013 and January 2021, consecutive patients who underwent elective aortoiliac revascularization were included in this prospective cohort study."
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page 7 (Paragraph 1): "Between January 2013 and January 2021, consecutive patients who underwent elective aortoiliac revascularization were included in this

			prospective cohort study. All patients were selected from a tertiary and a community hospital and had aortoiliac TransAtlantic Inter-Society Consensus (TASC) II type D lesions"
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up (*b*) For matched studies, give matching criteria and number of exposed and unexposed	Page 7 (Paragraph 1): "Between January 2013 and January 2021 (…) lesions, excluding those with aortoiliac aneurysmatic disease"
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page 8 (Paragraphs 2, 3): "The technical success of the operation was defined as (…) between postoperative (24h) and preoperative RDW-CV levels. "
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page 8 (Paragraph 4): "All patients had at least one sample of preoperative CBC available in the two weeks preceding the intervention. (…), with reference values for RDW-CV within 11% and 16%."
Bias	9	Describe any efforts to address potential sources of bias	N/A
Study size	10	Explain how the study size was arrived at	Page 9 (Paragraph 1): "The sample needed for a survival test was calculated applying WinPepi® V11.65, aiming for a statistical power (β) of 90% and an $\alpha < 0.05$. The sample was estimated (95 patients) for a hazard ratio of 2 between groups and a predicted survival at the end of follow-up of 80%."
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page 9 (Paragraph 3): "To ease the understanding and clinical use of the RDW-CV, the authors opted to

			establish a threshold. The best cut-off for RDW was obtained, resorting to an optimal binning."
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Page 9 (Paragraphs 2 and 4): "Student's t-test was favored when dealing with normally distributed (…) predictors of long-term MACE and all-cause mortality according to the backward stepwise regression method."
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) If applicable, explain how loss to follow-up was addressed	N/A
		(*e*) Describe any sensitivity analyses	N/A
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	Page 10 (Paragraph 1): "The cohort included a total of 107 participants, 95.3% male, with a median age of 62.2 ± 8.84 years."
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page 10 (Paragraph 1): "The cohort included a total of 107 participants, 95.3% male, with a median age of 62.2 ± 8.84 years. Thirty-eight patients had increased preoperative RDW-CV (35.5%). There were no significant differences in age or gender between the RDW-CV groups. The median follow-up of the cohort was 57 (95% CI 34.4-69.6) months" and Table I and II.
		(b) Indicate number of participants with missing data for each variable of interest	N/A
		(c) Summarise follow-up time (eg, average and total amount)	"The median follow-up of the cohort was 57 (95% CI 34.4-69.6) months."

Outcome data	15*	Report numbers of outcome events or summary measures over time	Table III and Figure 1
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included (*b*) Report category boundaries when continuous variables were categorized (*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page 10-12, Tabes I – III, Figures 1 and 2.
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page 12 (Paragraph 2): "This study found that an elevated preoperative RDW-CV can predict long-term outcomes such as AHF, stroke, MACE, and all-cause mortality in patients undergoing revascularization due to extensive aortoiliac disease. Additionally, Δ RDW-CV was an independent predictor of long-term all-cause mortality and MACE. No association was found regarding short-term outcomes."
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Page 14 (Paragraph 3): "There are some limitations inherent to this study (…) by several factors including not only gender but also age or ethnicity."
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page 12 (Paragraph 3): "As mentioned above, RDW-CV has been thoroughly (…) Absolute values of RDW and its reference intervals are influenced by several factors including not only gender but also age or ethnicity."
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page 15 (Paragraph 1): "On the other hand, the prospective

			nature of the design, (…) studies are necessary to confirm these findings."
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Page 19 (Paragraph 3): "This study has not received external funding"

*Give information separately for exposed and unexposed groups.
N/A – non applicable

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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- Standard article.

Liu H, Li J, Du L, Yang M, Yang D, Li J, et al. Short-term effects of core stability training on the balance and ambulation function of individuals with chronic spinal cord injury: a pilot randomized controlled trial. Minerva Med 2019;110:216-223

- Organization as author

International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Ann Int Med 1988;108:258-65.

- Both individual authors and organization as author

Castelli E, Fazzi E; SIMFER-SINPIA Intersociety Commission. Recommendations for the rehabilitation of children with cerebral palsy. Eur J Phys Rehabil Med. 2016;52:691-703.

- Issue with supplement

Lacarrubba F, Musumeci MI, Martorell A, Palmucci S, Petrillo G, Micali G. Role of the Imaging Techniques in the Diagnosis and Staging of Hidradenitis Suppurativa. G Ital Dermatol Venereol 2018;153 (3 Suppl 2), 20-5.

Books and monographs

For occasional publications, the names of authors, title, edition, place, publisher and year of publication must be given.

- Books by one or more authors

Rossi G. Manual of Otorhinolaryngology. Turin: Edizioni Minerva Medica; 1987.

- Chapter from book

Donas K, Torsello G. Management of Restenosis after Carotid Artery Stenting and Carotid Endarterectomy. In: Jacobs M (editor). Prevention and management of vascular complications. Turin: Edizioni Minerva Medica; 2011. p.17-20.

- Congress proceedings

Novo S, Angelides N, Fletcher J, Roztocil K, editors. A multidisciplinary approach to cardiovascular diseases. Proceedings of the 1st Meeting of the Multidisciplinary Chapter of the International Union of Angiology (IUA); 2014 Oct 2-5; Palermo, Italy. Turin: Edizioni Minerva Medica; 2016.

Electronic material

- Standard journal article on the Internet

Williams JS, Brown SM, Conlin PR. Videos in clinical medicine. Blood-pressure measurement. N Engl J Med. 2009 Jan 29;360(5):e6.

- Article published electronically ahead of the print version
Di Pierro F, Bertuccioli A, Cavecchia I, Possible therapeutic role of a highly standardized mixture of active compounds derived from cultured Lentinula edodes mycelia (AHCC) in patients infected with 2019 novel coronavirus. Minerva Gastroenterol Dietol 2020. [Epub ahead of print]
- Standard citation to a book on CD-ROM or DVD
Boglione L, Cariti G, Di Perri G. Interferon-free treatment of hepatitis C patients [CD-ROM]. Torino: Edizioni Minerva Medica; ©2017
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