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Original Research

Integrating Red Blood Cell Features and Hemoglobin Levels in Metastatic Renal Cell Carcinoma Patients Treated with Pazopanib or Cabozantinib: An Easily Exploitable Prognostic Score

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Abstract

Background: The advent of immune checkpoint inhibitors (ICIs) has revolutionized the metastatic renal cell carcinoma (mRCC) therapeutic landscape. Nevertheless, tyrosine-kinase inhibitors (TKIs) targeting the vascular endothelial growth factor (VEGF) axis still play a key role. The aim of the present study was to explore the prognostic performance of an integrated blood score, based on hemoglobin (Hb) concentration, mean corpuscular volume (MCV), and red cell distribution width (RDW), in mRCC patients treated with anti-VEGF TKIs. The primary endpoint was to correlate Hb, MCV, and RDW with progression-free survival (PFS) and overall survival (OS). **Materials and Methods**: Our multicenter retrospective observational study involved mRCC patients treated with pazopanib or cabozantinib from January 2012 to December 2020 in nine Italian centers. Clinical records and laboratory data, including Hb levels, MCV, and RDW, were collected at baseline. Descriptive statistics and univariate and multivariate analyses were performed. **Results**: We enrolled 301 mRCC patients of which 179 (59%) underwent pazopanib, and 122 (41%) cabozantinib. We considered baseline Hb $\geq$12 g/dL, MCV $>$87 fL, and RDW $\leq$16% as good prognostic factors; hence, developing a multiparametric score capable of delineating 4 different categories. The number of good prognostic factors was associated with significantly longer PFS and OS ($p < 0.001$ for both). Therefore, we developed a red blood cell-based score by stratifying cases into two groups (2–3 *versus* 0–1, good factors). The impact on PFS and OS was even more striking (median PFS (mPFS): 16.3 *vs* 7.9 months; median OS (mOS): 33.7 *vs* 14.1 months)), regardless of the TKI agent. When challenged with univariate and multivariate analysis, the blood score maintained its high prognostic significance in terms of OS (multivariate analysis HR for OS: 0.53, 95% CI 0.39–0.75; $p < 0.001$, respectively), while the impact on PFS resulted in borderline significance. **Conclusions**: Our analyses demonstrate the prognostic role of a multiparametric score based on easily exploitable blood parameters, such as Hb concentration, MCV, and RDW. The red blood cell-based score may underlie the upregulation of the HIF-1α pathway and VEGF axis, thereby identifying a selected population who is likely to benefit from TKI therapy.

Keywords: metastatic renal cell carcinoma (mRCC); macrocytosis; anisocytosis; anemia; HIF; tyrosine-kinase inhibitors; prognostic score; MCV; Hb; RDW

1. Introduction

The treatment landscape for metastatic clear cell renal cell carcinoma (mccRCC) is constantly evolving. Despite the recent advent of immunotherapy-based regimens, tyrosine kinase inhibitors (TKIs) still play a key role in mccRCC, except for sarcomatoid-differentiated subtypes [1,2], which are known to benefit more from immune-checkpoint inhibitors (ICIs). As a matter of fact, the combination of ICIs with TKIs represents a valid option for all intermediate and poor-risk patients according to the International Metastatic Renal Cell Carcinoma Database Consortium (IMDC) score, especially when a timely disease control is needed [3]. Various combinations have gained U.S. Food and Drug Administration (FDA) and European Medicines Agency (EMA) approval for clinical use in this setting: pembrolizumab plus axitinib (KEYNOTE 426 trial), pembrolizumab plus lenvatinib (CLEAR trial), avelumab plus axitinib (JAVELIN Renal 101 trial), and nivolumab plus cabozantinib (CheckMate 9ER trial) [4–7]. Moreover, TKIs may represent a monotherapy option in favorable-risk patients presenting low tumor burden and indolent disease progression patterns, or in selected cases who cannot receive ICIs [8]. Sunitinib, an oral TKI, which inhibits different growth factors, such as vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), and c-KIT, was the first TKI to show a significant benefit in progression-free survival (PFS) and overall survival (OS), compared to the current standard of care treatment with interferon-α [9]. Of note, two further multitarget TKIs are currently used in the metastatic setting: pazopanib and cabozantinib. Pazopanib inhibits VEGFR, PDGFR, fibroblastic growth factor receptor (FGFR), c-KIT, and RET, and has now been approved for first-line therapy in metastatic renal cell carcinoma (mRCC) [10], while cabozantinib, given its marked activity across VEGFR2, mesenchymal-epithelial transition factor (MET), c-KIT, and tyrosine-protein kinase receptor UFO (AXL), represents a valid therapeutic option for intermediate/poor-risk patients in the first-line setting [11], and in further lines following antiangiogenic therapy and irrespective of IMDC risk [12].

This class of drugs mainly works by blocking the hypoxia-inducible factor-1-alpha (HIF-1α) downstream pathways, particularly the VEGF axis, which is involved in several processes, including metabolic adaptation, angiogenesis, cell growth, differentiation, and survival; thus, making it critical in RCC development and progression [13]. HIF-1α is an oxygen-sensitive subunit of HIF-1, the latter being a heterodimeric transcription factor, which is responsible for the adaptive response of tumor cells to hypoxic conditions through the transcriptional activation of over 100 genes. Unlike HIF-1β, which is the constitutively expressed subunit of HIF, HIF-1α is induced by hypoxia and enables the heterodimerization and subsequent activation of the HIF-1 complex. In normoxic conditions, HIF-1α is promptly degraded, mostly via ubiquitination and proteasomal degradation mediated by the von Hippel-Lindau protein (pVHL) [14,15].

The loss of the *VHL* gene is an early tumorigenesis event that occurs in approximately 90% of ccRCCs, which leads tumor cells to a state of pseudohypoxia, and consequently, upregulates HIF-1α expression and the activation of its downstream genes [16].

In addition to the VEGF axis, one of the main target genes of the HIF-1 complex is erythropoietin (EPO). This hormone, mostly synthesized in the renal cortex by peritubular fibroblasts, is responsible for keeping the blood hemoglobin (Hb) concentration within a normal range by promoting survival, proliferation, and differentiation of erythrocytic progenitors [17].

Moreover, according to both Memorial Sloan Kettering Cancer Center (MSKCC) and IMDC prognostic scores, anemia is negatively associated with mRCC survival [18, 19]. Interestingly, it is ranked amongst the adverse events related to anti-VEGF TKIs and it seems to be related to off-target effects, such as the inhibition of FLT-3 and c-KIT, especially during treatment with sunitinib [20]. Nonetheless, increases in Hb concentration and red blood cell counts in the blood have also been reported during treatment with these agents [21–23].

Interestingly, the elevation of the mean corpuscular volume (MCV), which reflects macrocytosis, is another phenomenon described during treatment with anti-VEGF TKIs [24,25] and correlates with a survival benefit in mRCC patients treated with sunitinib [26–28].

Furthermore, among red blood cell-centered parameters, red cell distribution width (RDW)—an indirect measure of anisocytosis—has been proven to be clinically meaningful, being directly associated with the grading and staging of the disease in localized RCCs, in addition to cancer-specific mortality in mRCC patients who underwent partial or total nephrectomy [29,30]. Of note is a study by Aktepe *et al.* [31], which revealed that a higher RDW level correlated with a shorter PFS and OS in mRCC patients treated with either sunitinib or pazopanib.

Our preliminary data revealed that a relevant proportion of patients with mRCC treated with pazopanib or cabozantinib had baseline macrocytosis or anisocytosis. Moreover, we demonstrated a significant increase in Hb, MCV, and RDW values following TKI treatment. Baseline macrocytosis resulted in a positive correlation with PFS in patients treated with pazopanib, while anisocytosis emerged as a negative prognostic factor for all patients treated with pazopanib or cabozantinib. The evidence provided by this study suggests that Hb, MCV, and RDW may indirectly reflect the activation of the HIF-1α pathway in patients with mRCC.

Thus, the aim of the present study was to delineate a new integrated prognostic score in mRCCs treated with anti-VEGF TKIs, based on easily exploitable blood param-

eters, such as Hb concentration, MCV (macrocytosis), and RDW (anisocytosis), which may reflect an upregulation of the HIF-1α pathway, and subsequently VEGF axis, thereby potentially identifying a selected population who can most benefit from TKI therapy.

2. Materials and Methods

Our multicenter observational retrospective study was conducted on patients with mRCC who were undergoing TKI treatment with pazopanib or cabozantinib between January 2012 and December 2020 in nine Italian centers. The primary endpoint of the study was to assess the impact of our new integrated prognostic score, based on Hb concentration, MCV, and RDW, on PFS and OS.

2.1 Patient Population

Our study cohort involved patients with unresectable or metastatic RCCs, histologically confirmed, who had received pazopanib or cabozantinib at any time in their treatment.

Clinicopathological records, including Eastern Cooperative Oncology Group Performance Status (ECOG PS), prognostic score using the International mRCC Database Consortium (IMDC) criteria, metastatic involvement, histopathological characteristics, and hematological/biochemical parameters (MCV, RDW, and Hb levels), were collected at baseline, before starting TKI treatments. Exclusion criteria consisted of systemic treatment other than cabozantinib or pazopanib and an absence of medical records.

The study was conducted following the approval by the ethics committee of the coordinating Center (protocol number 208/2021/OSS/AOUPR MA.RE.CA.P., date of approval: September 1, 2021) and the obtainment of patient informed consent.

2.2 Statistics

Descriptive statistics were employed to report on patient characteristics. Receiver operating characteristics curves (ROC)-based cut-offs were used to select the optimal values of MCV ($>$87 fL: macrocytosis) and RDW ($\leq$16%: anisocytosis) able to maximize the log-rank test, while anemia was defined as a hemoglobin (Hb) value lower than 12 g/dL.

The PFS was intended as the time from the beginning of the TKI therapy to the disease progression or death, whichever occurred first. The OS was calculated from the start of treatment to death for any cause. Patients without progression or death at the last follow-up were considered censored. Median follow-up was calculated according to the so-termed "reverse Kaplan–Meier" (Kaplan–Meier estimate of potential follow-up) technique. PFS and OS were estimated using the Kaplan–Meier method, while the log-rank test (Mantel–Cox) was applied to evaluate statistical differences in PFS and OS between groups. Then, PFS and OS data were analyzed through Cox univariate and multivariate proportional hazards regression models and the results were expressed as hazard ratios (HR), 95% confidence intervals (95% CI), and p values. The multivariate models were fitted to include covariates which resulted significant in the univariate analysis. To minimize the risk of multiplicity, Holm–Bonferroni correction test was applied to all multi-variant comparisons. The threshold for statistical significance was set to a p value of 0.05. IBM SPSS Statistics v. 25.0 (IBM SPSS Statistics for Windows, Version 25.0, IBM Corp., Armonk, New York, USA) was used to perform all the computational analyses. JAMOVI version 2.3.21 (https://www.jamovi.org/) was used for drawing the survival curves.

The preplanned analyses were computed in the overall patient population and within each treatment group (pazopanib or cabozantinib).

3. Results

3.1 Patient Characteristics

During the study period, we enrolled 301 mRCC patients: 179 (59%) underwent pazopanib, while 122 (41%) were treated with cabozantinib. Baseline clinicopathological characteristics in the overall population and TKI subgroups are reported in Table 1. The median age was 68 years, with a clear prevalence of males over females (68% *vs* 32%). According to IMDC criteria, 53% of patients belonged to the intermediate prognostic group, while 95% of patients had an ECOG PS of 0 or 1. The vast majority of patients underwent a nephrectomy (85%). In regard to the sites of the metastases, the lung was the most common (64%), followed by nodes (42%), and bones (37%), while glands and liver accounted for 19% each. Pazopanib was administered as the first-line treatment in 97% of cases, while cabozantinib was mainly administered as second (42%) or further-line therapy (44%). The mean Hb value was 12.5 g/dL, while about two-thirds of patients presented baseline macrocytosis (MCV levels $>$87 fL), and 45% had baseline anisocytosis (RDW $>$16%).

In the overall population, the median PFS (mPFS) and OS (mOS) were 12.0 (95% CI 9.5–14.6) and 25.8 months (95% CI 21.3–30.2), respectively.

3.2 Impact of Multiparametric Score on Survival Outcome

Based on our preliminary data, which strongly suggested positive prognostic values of Hb $\geq$12 g/dL (absence of anemia), MCV $>$87 fL (macrocytosis), and RDW $\leq$16% (absence of anisocytosis), we generated a multiparametric score. According to the presence of 0, 1, 2, or 3 good prognostic factors, we delineated 4 different groups. Prolonged PFS was observed in patients presenting 2 (mPFS 14.7 months, 95% CI 7.5–21.9) or 3 (mPFS 16.6 months, 95% CI 14.3–18.8) good factors compared to 0 (mPFS 8.2 months, 95% CI 5.2–11.2) or 1 (mPFS 7.7 months, 95% CI 3.8–11.6) in the subgroups ($p < 0.001$, Fig. 1A). Similar evidence also emerged in terms of median OS (mOS), which was meaningfully longer in cases displaying 2 or 3 good factors ($p < 0.001$, Fig. 1B).

Table 1. Clinicopathological characteristics.

Number of patients (%)	Overall	Pazopanib group	Cabozantinib group
	301 (100%)	179 (59%)	122 (41%)
Median age (range)	68 (36–89)	70 (42–89)	65 (36–85)
Sex (%)			
Male	206 (68.4)	126 (70.4)	80 (65.6)
Female	95 (31.6)	52 (29.4)	42 (34.4)
Histology (%)			
Clear cell	250 (83.1)	152 (84.9)	98 (80.3)
Papillary	24 (8.0)	11 (6.1)	13 (10.7)
Chromophobe	8 (2.7)	5 (2.8)	3 (2.5)
NOS	19 (6.3)	11 (6.1)	8 (6.6)
IMDC score (%)			
Good	103 (34.2)	65 (36.3)	38 (31.1)
Intermediate	159 (52.8)	92 (51.4)	67 (54.9)
Poor	39 (13.0)	22 (12.3)	17 (13.9)
ECOG PS (%)			
0	183 (60.8)	10 (61.5)	73 (59.8)
1	102 (33.9)	61 (34.1)	41 (33.6)
2–3	16 (5.4)	8 (4.5)	8 (6.5)
NLR (%)			
<3	183 (60.8)	80 (44.7)	43 (35.2)
≥ 3	102 (33.9)	75 (41.9)	65 (53.3)
NA	38 (12.6)	24 (13.4)	14 (11.5)
Nephrectomy (%)			
Yes	256 (85)	149 (83.2)	107 (87.7)
No	45 (15)	30 (16.8)	15 (12.3)
Median number of metastatic sites (range)	2 (1–8)	2 (1–6)	3 (1–8)
Sites of metastasis (%)			
Lung	194 (64.5)	116 (64.8)	78 (63.9)
Liver	58 (19.3)	29 (16.2)	29 (23.8)
Nodes	126 (41.9)	58 (32.4)	68 (55.7)
Bone	112 (37.2)	53 (29.6)	59 (48.4)
Glands	58 (19.3)	30 (33.5)	28 (23.0)
Other	114 (37.9)	60 (33.5)	54 (44.3)
Use of PPI (%)			
Yes	132 (43.9)	69 (38.5)	63 (51.6)
No	169 (56.1)	110 (61.5)	59 (48.4)
Line of treatment (%)			
1st	192 (63.8)	175 (97.8)	17 (13.9)
2nd	54 (17.9)	3 (1.0)	51 (41.8)
$\geq$3rd	55 (18.3)	1 (0.2)	54 (44.2)
Hb values			
<12 g/dL	121 (40.2)	61 (34.1)	60 (49.2)
≥ 12 g/dL	180 (59.8)	118 (65.9)	62 (50.8)
MCV			
<87 fL	102 (33.9)	68 (38.0)	34 (27.9)
≥ 87 fL	199 (66.1)	111 (62.0)	88 (72.1)
RDW			
≤ 16	164 (54.5)	108 (60.3)	57 (46.7)
>16	137 (45.5)	71 (39.7)	65 (53.3)

Abbreviations: NOS, not otherwise specified; IMDC score, International Metastatic RCC Database Consortium Score; ECOG PS, Eastern Cooperative Oncology Group Performance Status; NLR, neutrophil-to-lymphocyte ratio; NA, not available; PPI, proton pump inhibitor; Hb, hemoglobin; MCV, mean corpuscular volume; RDW, red cell distribution width.

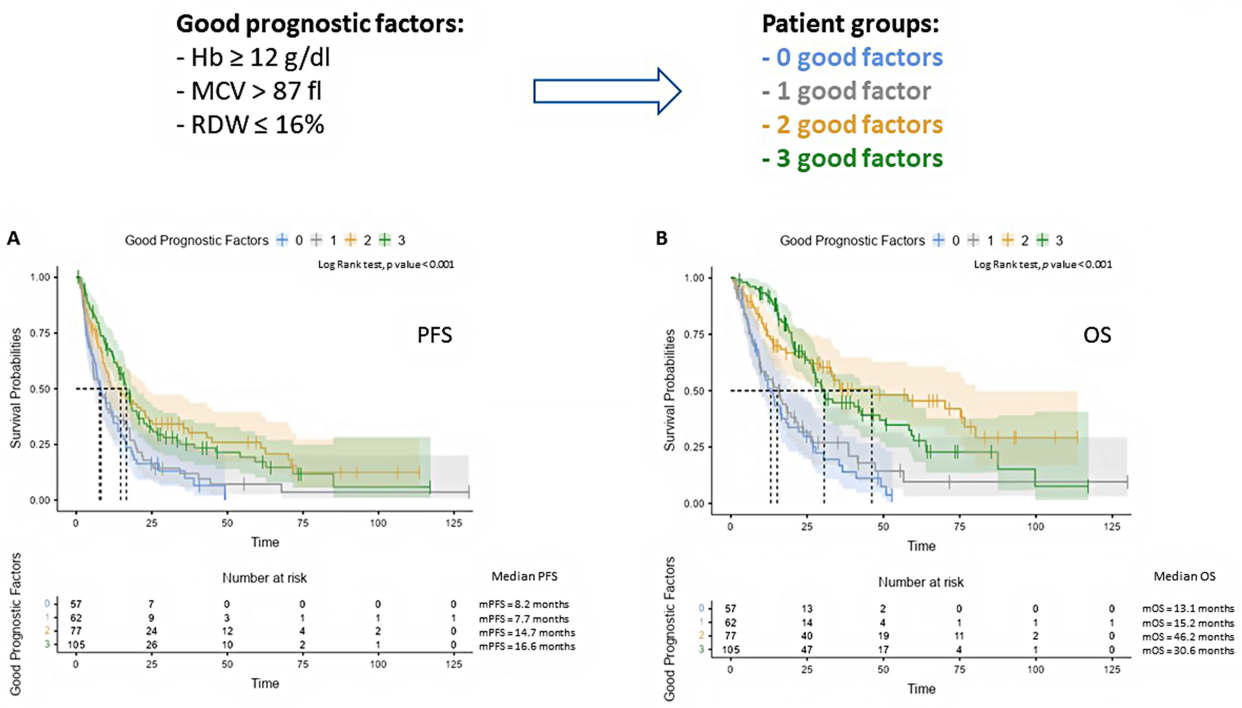

Fig. 1. Representative Kaplan–Meier curves illustrating the impact of the number of good prognostic factors on patient PFS (A) and OS (B). Number at risk is reported at the bottom of the curve. Hb, hemoglobin; MCV, mean corpuscolar volume; RDW, red cell distribution width; OS, overall survival; PFS, progression-free survival.

Significance was preserved for both PFS and OS in pazopanib-treated patients (**Supplementary Fig. 1A*i*** and **A*ii***), while only for OS in cabozantinib-treated mRCC (**Supplementary Fig. 2A*i*** and **A*ii***), after stratifying patients by TKI agents

In view of this preliminary finding, we developed a red cell-based "blood score" that allowed us to split our patient population into two categories: favorable (2–3 good factors) and unfavorable (0–1 good factors). Thereafter, the impact on PFS and OS was even more remarkable (mPFS 16.3 (95% CI 13.4–19.1) *vs* 7.9 (95% CI 5.3–10.4) months; mOS 33.7 (95% CI 23.9–43.4) *vs* 14.1 (95% CI 10.3–17.9) months), as clearly highlighted in Fig. 2A,B.

When we separately analyzed the outcome of the pazopanib and cabozantinib populations, the prognostic value of our integrated score was maintained in both subgroups. Specifically, in the pazopanib-treated subgroup presenting 2–3 good factors (favorable group), where the median PFS (17.7 months, 95% CI 13.8–21.6), and OS (46.2 months 95% CI 10.4–17.8) were significantly longer compared to the unfavorable group ($p < 0.001$, **Supplementary Fig. 3A*i*** and **A*ii***). Likewise, among mRCC patients receiving cabozantinib, the favorable group presented significantly prolonged PFS (mPFS 14.0 months, 95% CI 9.9–18.1) and OS (mOS 26.1 months, 95% CI 18.2–34.0) durations compared to the unfavorable group ($p = 0.012$ and <0.001, respectively; **Supplementary Fig. 4A*i*** and **A*ii***).

To further confirm the prognostic impact of our integrated score, we applied univariable and multivariable regression models that, in addition to our multiparametric score, encompassed predetermined key covariates: sex (male *vs* female), ECOG-PS (0 *vs* ≥1), histology (clear cell *vs* papillary *vs* chromophobe *vs* other), IMDC risk group (good *vs* intermediate *vs* poor), neutrophil-to-lymphocyte ratio (NLR) (<3 *vs* ≥3), bone metastases (yes *vs* no), liver metastases (yes *vs* no), proton pump inhibitors (PPIs) use (yes *vs* no), and nephrectomy (yes *vs* no).

As reported in Table 2, in the univariate analysis, ECOG PS >0, non-clear cell histology (chromophobe, papillary), intermediate-poor IMDC groups, NLR ≥3, liver and bone metastases, PPI use, and unfavorable blood score (0–1 good factors) were all able to condition a shorter PFS. In regard to OS, all the aforementioned characteristics preserved their significance, except for liver and bone metastases and histology (Table 3).

When challenged in the multivariate model, the prognostic value of histology, IMDC, NLR, liver metastases, and proton pump inhibitor (PPI) use was confirmed, in terms of PFS (Table 2), while only IMDC, NLR, and PPI use reached statistical significance for OS (Table 3). Of note, the blood score retained its highly significant impact on OS (HR 0.53, 95% CI 0.39–0.75, $p < 0.001$), while being borderline significant in terms of PFS (HR for PFS 0.74, 95% CI 0.55–1.02, $p = 0.069$).

4. Discussion

TKIs are currently recommended both as first-line (alone or in combination with ICIs) and further lines of treatment in mRCC; however, only a portion of mRCC pa-

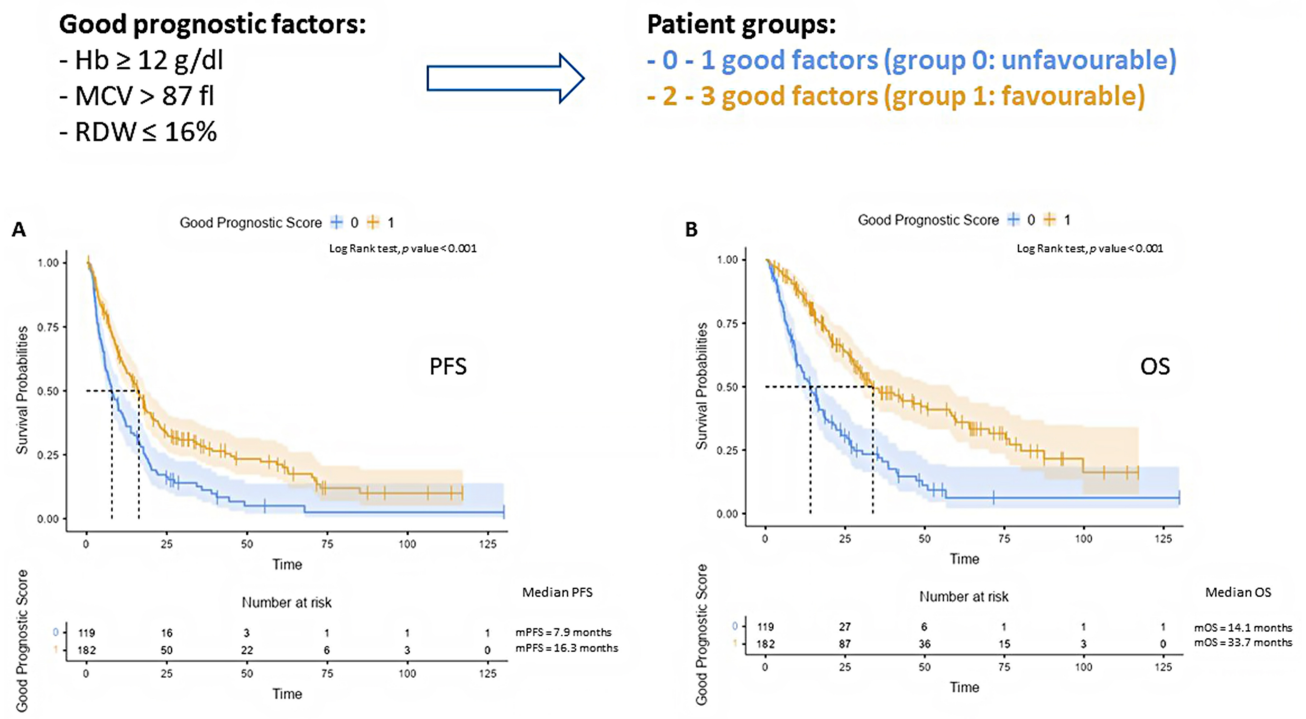

Fig. 2. Representative Kaplan–Meier curves illustrating the impact of our red blood cell-based score on patient PFS (A) and OS (B). Number at risk is reported at the bottom of the curve.

tients are able to gain a meaningful benefit, meaning the prediction of any long-term responses to these treatments remains a challenging and unsolved issue [32,33]. Therefore, effective and easily exploitable predictive markers are required for individual clinical trial design and patient management. Thus, we aimed to unveil the potential prognostic role of a multiparametric blood score, which accounts for predetermined features, such as baseline Hb concentration, MCV, and RDW, in mRCC patients treated with anti-VEGF TKIs.

Among tumor- and patient-specific parameters, the reliability of anemia, a well-established risk factor in the MSKCC score developed during the cytokine era [18], has been extensively investigated in the context of the TKI-driven therapeutic landscape. Indeed, evidence has been presented numerous times indicating that serum hemoglobin below the lower limit of normal (LLN) was a meaningful predictor for shorter OS and PFS [34–37]. Conversely, the prognostic significance of hemoglobin changes following TKI treatments is still debated and under intense scrutiny. A number of studies reported a transient increase in hemoglobin levels in 23.8% to 90% of mRCC patients, which peaked at 4–9 weeks after the onset of treatment [38,39]. Similarly, our preliminary observations on 301 mRCC patients undergoing TKIs confirmed a significant rise in blood Hb concentrations, with a mean increase of 1 g/dL, as early as day 15. The underlying mechanism might reside in the reinforcement of the HIF-1α/EPO pathway, induced by the downstream inhibition of VEGFR and PDGFR (Fig. 3).

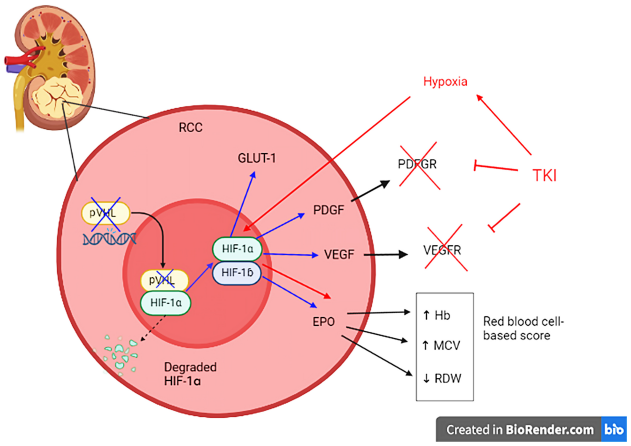

Fig. 3. Explanatory figure illustrating the HIF-α based mechanism supporting our data. RCC, renal cell carcinoma; TKI, tirosine kinase inhibitors; HIF, hypoxia inducible factor-1a; EPO, eritropoietin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PDGF, platelet-derived growth factor; PDGFR, platelet-derived growth factor receptor; GLUT-1, glucose transporter 1; pVHL, von Hippel–Lindau protein.

A few investigations have also demonstrated that increased hemoglobin may be associated with longer survival [38,40–42]. Conversely, despite the limitation of including small sample size and lack of correction for well-known clinical prognostic factors, a retrospective analysis by Tripathi *et al.* [43] documented significantly shorter time to

Table 2. Explanatory prognostic factors in univariate and multivariable Cox proportional hazard models.

PFS	Univariate			Multivariate		
	HR	95% CI	Adj. *p* value	HR	95% CI	Adj. *p* value
Sex			$p = 0.073$			
Male	ref					
Female	0.77	0.59–1.02				
ECOG PS			$p = 0.014$			$p = 0.904$
0	ref			ref		
≥1	1.48	1.08–1.85		1.06	0.76–1.48	
Histology			$p = 0.028$			$p = 0.016$
Clear Cell	ref			ref		
Papillary	1.58	0.98–2.55		1.58	0.93–2.68	
Chromophobe	2.53	1.24–5.14		2.81	1.34–5.89	
Other	0.67	0.38–1.17		0.88	0.48–1.64	
IMDC			$p = 0.01$			$p < 0.001$
Good	ref			ref		
Intermediate	1.08	0.81–1.43		0.93	0.68–1.28	
Poor	2.95	1.99–4.37		2.25	1.44–3.52	
NLR			$p = 0.014$			$p = 0.028$
<3	ref			ref		
≥3	1.55	1.18–2.04		1.38	1.03–1.83	
Liver metastasis			$p = 0.042$			$p = 0.043$
No	ref			ref		
Yes	1.45	1.06–1.99		1.43	1.01–2.02	
Bone metastasis			$p = 0.028$			$p = 0.060$
No	ref			ref		
Yes	1.43	1.10–1.85		1.32	0.99–1.77	
Use of PPIs			$p = 0.01$			$p = 0.018$
No	ref			ref		
Yes	1.55	1.20–2.205		1.42	1.06–1.89	
Nephrectomy			$p = 0.014$			$p = 0.939$
No	ref			ref		
Yes	0.59	0.42–0.82		1.02	0.67–1.54	
Red blood cell-based score			$p = 0.01$			$p = 0.069$
0–1 factors (unfavorable group)	ref			ref		
2–3 factors (favorable group)	0.56	0.43–0.72		0.74	0.55–1.02	

Abbreviations: 95% CI, 95% confidence intervals; PFS, progression-free survival; ECOG PS, Eastern Cooperative Oncology Group Performance Status. IMDC, International mRCC Database Consortium criteria; NLR, neutrophil-to-lymphocyte ratio; PPIs, proton pump inhibitors.

Adjusted *p* values following Holm–Bonferroni post-hoc test. HR, Hazard ratio; ref, reference.

treatment failure (TTTF) and PFS in patients displaying an increase in Hb levels.

Concerning the role of macrocytosis in mRCC patients, it has been repeatedly reported that its onset following TKI treatment correlated to a better survival outcome [26–28]. This evidence may be linked with the role of c-KIT-mediated signaling which, upon TKI inhibition, could determine an impaired maturation of blood elements derived from c-KIT expressing hematopoietic stem cells, and the consequent release of larger erythrocytes into the bloodstream [25].

Finally, among red blood cell-centered features, the presence of anisocytosis was negatively associated with mRCC survival, as effectively demonstrated in a retrospective observational study that included patients treated with sunitinib or pazopanib [31].

In the current investigation, we first revealed the clinical impact of combining Hb levels, MCV, and RDW on mRCC patient outcomes. Thereafter, we developed a multiparametric blood score integrating all the above-mentioned factors and delineating two prognostic categories. Specifically, patients carrying at least 2 good prognostic factors (Hb ≥12 g/dL and/or MCV >87 fL and/or RDW ≤16%) exhibited significantly prolonged PFS and OS compared to the unfavorable group (0–1 good prognostic factors). Notably, when tested by multivariate analysis, including

Table 3. Explanatory prognostic factors in univariable and multivariable Cox proportional hazard models.

OS	Univariate			Multivariate		
	HR	CI (95%)	Adj. *p* value	HR	CI (95%)	Adj. *p* value
Sex			$p = 0.192$			
Male	ref					
Female	0.74	0.53–1.02				
ECOG PS			$p = 0.01$			$p = 0.725$
0	ref			ref		
≥1	1.68	1.26–2.24		1.04	0.77–1.40	
Histology			$p = 0.192$			
Clear Cell	ref					
Papillary	1.70	1.02–2.82				
Chromophobe	1.67	0.78–3.56				
Other	0.73	0.38–1.39				
IMDC			$p = 0.01$			$p < 0.001$
Good	ref			ref		
Intermediate	1.61	1.14–2.25		0.12	1.35–0.92	
Poor	5.78	3.72–8.99		4.14	2.50–6.88	
NLR			$p = 0.01$			$p = 0.014$
<3	ref			ref		
≥3	1.73	1.26–2.36				
Liver metastasis			$p = 0.192$			
No	ref					
Yes	1.30	0.91–1.86				
Bone metastasis			$p = 0.06$			
No	ref					
Yes	1.44	1.07–1.93				
Use of PPIs			$p = 0.01$			$p = 0.005$
No	ref			ref		
Yes	1.67	1.25–2.22		1.58	1.51–2.16	
Nephrectomy			$p = 0.01$			$p = 0.920$
No	ref			ref		
Yes	0.46	0.32–0.65		0.97	0.62–1.53	
Red blood cell-based score			$p = 0.01$			$p < 0.001$
0–1 factors (unfavorable group)	ref			ref		
2–3 factors (favorable group)	0.38	0.29–0.51		0.53	0.39–0.75	

Abbreviations: 95% CI, 95% confidence intervals; PFS, progression-free survival; ECOG PS, Eastern Cooperative Oncology Group Performance Status. IMDC, International mRCC Database Consortium criteria; NLR, neutrophil-to-lymphocyte ratio; PPIs, proton pump inhibitors.

Adjusted *p* values following Holm–Bonferroni post-hoc test. HR, Hazard ratio; ref, reference.

clinico-pathological covariates, known to be robust prognostic factors for patients with mRCC (i.e., sex, ECOG PS, histology, IMDC group, NLR, PPI assumption, bone metastases, liver metastases, and nephrectomy), the red blood cell score preserved its clinical relevance in terms of OS (HR 0.53, 95% CI 0.39–0.75, $p < 0.001$), while being at borderline significance in terms of PFS (HR 0.74, 95% CI 0.55–1.02, $p = 0.069$). In this regard, it is worth highlighting that the concomitant assumption of PPIs, a class of drugs with well-known effects on MCV, and likely conditioning mRCC therapeutic outcome under TKIs [44], did not affect the prognostic performance of our score, thereby strengthening our data.

Based on these findings, we hypothesized that elevated Hb, macrocytosis, and low anisocytosis may be sustained by EPO stimulation depending on HIF-1α pathway upregulation. The enhanced activity of the HIF-1α axis is a hallmark of mRCC and constitutes the rationale for the therapeutic use of VEGF-TKIs. As exemplified in Fig. 3, we speculated that TKI agents, by inhibiting VEGFR and PDGFR targets downstream, may boost the HIF-1α/EPO signaling pathway, and this may be even more relevant since that particular tumor requires activation of the HIF pathway for its own growth. This hypothesis is corroborated by literature evidence demonstrating that erythrocytosis, secondary to anti-VEGF treatment, was a distinc-

tive feature of mRCC patients, which has not been documented in patients with other malignancies treated with the same agents. This phenomenon suggests that anti-VEGF-induced elevated EPO levels might be connected to RCC itself [21]. Accordingly, red blood cells displaying high MCV and low RDW likely reflect the presence of reticulocytes in the bloodstream, in response to increased levels of EPO.

In spite of the intrinsic limitation of a retrospective nature, the multicenter involvement, the adequate median follow-up, the balanced TKI type, and the treatment line, represent strengths in our study. Moreover, the high prognostic performance of our red blood cell score likely resided in its multiparametric nature, is independent of the TKI drugs and might be effectively exploitable in clinical practice since the proposed circulating parameters could be easily obtained by a "simple" blood sample.

5. Conclusions

To the best of our knowledge, the present work is the first retrospective observational investigation to provide evidence on the clinical relevance and applicability of a multiparametric blood score based on hemoglobin levels, MCV, and RDW values and can be used to identify mRCC patients who might gain benefit from TKI therapy (cabozantinib or pazopanib).

In-depth analyses aimed at assessing circulating EPO and erythroblasts that will corroborate our hypothesis are currently ongoing.

Future studies are warranted to prospectively test the validity of our score in mRCC patients treated with immune combinations.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

GM: conceptualization, project administration, visualization, writing – original draft, writing: review & editing; AL: data curation, writing – original draft; MSa: data curation, review, editing; FT: data curation, writing – original draft; UDG: data curation, review, editing; NB: data curation, review, editing; CT: data curation, review, editing; SP: data curation, review, editing; OC: data curation, review, editing; SK: data curation, review, editing; AMe: data curation, review, editing; CC: data curation, review, editing; EV: data curation, review, editing; AR: data curation, review, editing; MSt: data curation, review, editing; AMa: data curation, review, editing; GR: data curation, review, editing; EMS: data curation, review, editing; PR: data curation, review, editing; SER: data curation, review, editing; GF: data curation, review, editing; GCG: visualization, data curation, review, editing; GLB: data curation, critical review, editing; FQ: data curation, review, editing; SB: conceptualization, data curation, formal analysis, methodology, project administration, visualization, writing – original draft, writing: review & editing. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The study was conducted following the approval by the ethics committee of the coordinating Center (protocol number 208/2021/OSS/AOUPR MA.RE.CA.P., date of approval: September 1, 2021) and the obtainment of patient informed consent.

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Conflict of Interest

Sebastiano Buti received honoraria as a speaker at scientific events and advisory role by Bristol-Myers Squibb (BMS), Pfizer; MSD, Ipsen, AstraZeneca and Novartis; he also received research funding from Novartis, but we can confirm that these grants do not interfere at all with this manuscript and the presented data. The other authors have no conflicts of interest to disclose.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/j.fbe1503020.

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