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Carfilzomib-induced hemolysis is noticeably common but rarely shows features of thrombotic microangiopathy: A retrospective study

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Abstract

Objective: Hemolysis is a sporadically reported but potentially serious side effect of the proteasome inhibitor carfilzomib. We aimed to investigate the frequency of hemolysis in an unselected cohort.

Methods: We performed a retrospective, single-center study of the incidence of hemolysis in patients treated with carfilzomib, based mainly on consecutive haptoglobin levels. The patients were diagnosed with myeloma (n = 20), AL amyloidosis (n = 3), and light-chain deposition disease (n = 1). Carfilzomib treatment was applied after a median of 3 (range: 1-7) therapy lines.

Results: Haptoglobin levels were normal/increased before, generally suppressed during, and normalized after treatment with carfilzomib. Very low haptoglobin (<0.1 g/L) implying the presence of hemolysis was observed in 16 of 24 (67%) patients during carfilzomib therapy. Hemolysis was mild in 11 of 16 (69%) affected patients, whereas 5 of 16 (31%) required transfusion. Severe hemolysis was explained by thrombotic microangiopathy (TMA) in one patient who died of the complication. Mechanisms were unclear in the remaining 15 patients.

Conclusions: Hemolysis was surprisingly common but mostly mild during carfilzomib treatment. However, the possibility of TMA should be kept in mind in this setting. Hypothetically, non-TMA hemolysis could be attributed to the accumulation of globin chains due to the suppression of eukaryotic translation initiation inhibition by carfilzomib.

KEYWORDS

hemolysis, neoplasia-myeloma and other plasma cell dyscrasias, proteasome inhibitors, thrombotic microangiopathies

1 | INTRODUCTION

Carfilzomib (CFZ) is an irreversible second-generation proteasome inhibitor used for the treatment of plasma cell dyscrasias such as multiple myeloma[1,2] and recently light-chain (AL) amyloidosis.[3] Anemia is a common side effect of CFZ[1,2] although its mechanism is unclear. Hemolysis has not been reported in clinical prospective studies with CFZ.[1,2] However, microangiopathic hemolytic anemia

(MAHA) as the hallmark of thrombotic microangiopathy (TMA) has been described in single patients treated with CFZ[4-7] and in case series,[8-11] the latter also including patients treated with bortezomib.[9] Besides MAHA, CFZ-induced TMA presented commonly with renal failure and hypertension in addition to thrombocytopenia.[4-11] Recently, mostly well-compensated hemolysis was observed in 8 of 10 patients treated with CFZ in a single center.[12] The most sensitive and specific hemolysis parameter is a decreased plasma level of the heme scavenger protein haptoglobin (Hp).[13] Here, we aimed to explore the frequency with which hemolysis occurs based on Hp levels in patients receiving CFZ, and to identify patient and treatment characteristics associated with hemolysis occurrence.

2 | PATIENTS AND METHODS

We studied all patients with plasma cell dyscrasias treated with CFZ at Örebro University Hospital between October 2015 and April 2019. The patients were identified through the hospital registry and had diagnoses of myeloma (n = 20), AL amyloidosis (n = 3), and light-chain deposition disease (n = 1). Clinical and laboratory data were collected retrospectively from medical records. Hp analysis is a part of the protein panel assessment used for laboratory follow-up of plasma cell dyscrasias at our institution. Hp was measured using turbidimetry with polyclonal rabbit anti-human haptoglobin (Dako), with the reference range of 0.22-1.9 g/L. Hp values lower than 0.1 g/L were not specified by the laboratory (reported as <0.1 g/L) and were regarded as indicative of hemolysis. CFZ side effects were assessed according to Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.[14] Date of the last follow-up of the survivors was April 2, 2019. The study was approved (reference 2011/356) by the Regional Ethical Review Board in Uppsala, Sweden, in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients. Patient and treatment characteristics were compared with hemolysis occurrence using univariate logistic regression. Statistical significance was set as P value < .05. Analyses were performed using StataCorp. 2017. *Stata Statistical Software: Release 15*. College Station, TX: StataCorp LLC.

3 | RESULTS

A total of 24 patients with a median age of 66 (range: 47-78) years at the start of CFZ treatment were included. Patient characteristics and details of CFZ therapy are presented in Table 1 and Figure 1. The median number of prior treatment lines was three (range: 1-7), which included bortezomib in 21 of 24 (88%) and lenalidomide in 19 of 24 (79%) patients. One patient had undergone an allogeneic stem cell transplantation (SCT), whereas 15 of 24 (63%) had undergone autologous SCT. CFZ was given intravenously, together with oral dexamethasone, on days 1, 2, 8, 9, 15, and 16 of a 28-day cycle. In 21 of 24 patients (88%), paraprotein levels decreased initially during the CFZ treatment (formal response criteria were not utilized). Until the date of last follow-up, 21 patients terminated CFZ treatment due to achieved treatment goal (n = 3), second autologous SCT (n = 4), disease progression (n = 6), cardiopulmonary adverse effects (n = 3), hemolytic anemia (n = 1), TMA (n = 1), and death (n = 3, caused by myeloma, myocardial infarction, and pulmonary edema, respectively). Median survival from the start of CFZ was 10.5 (range: 1-40) months, and in total, 9 of 24 patients (38%) died during follow-up.

During the period of CFZ treatment and two to three months before/after treatment, Hp was measured with a median interval of 28 (range: 1-64) days. Trajectory analysis revealed that Hp levels were normal/increased in all patients before the start of treatment (Figure 2). Hp levels generally decreased during treatment with CFZ (Figure 2) and became very low (<0.1 g/L) in 16 of 24 (67%) patients. In 9 patients, Hp levels were continuously very low during the remaining CFZ treatment period. Median time from the start of CFZ treatment until hemolysis occurred (Hp < 0.1 g/L) was 52 (range: 21-188) days (Figure 1). In all patients available for follow-up, Hp levels returned to normal after CFZ was discontinued (Figure 2).

Other hemolysis parameters were assessed in 14 patients by the treating physicians after noticing very low Hp, with at least one parameter being elevated in 13 (93%) and at least two in 9 (64%) of the patients. Specifically, lactate dehydrogenase was elevated in 9 of 14 (64%), reticulocytes in 10 of 13 (71%), and total bilirubin in 4 of 14 (28%) tested patients. Hemolysis was graded (according to CTCAE) as 1-2 in 11 of 16 (69%) and 3-4 (transfusion required) in 5 of 16 (31%) affected patients (Figure 1). Occurrence of hemolysis was more common in patients receiving more than 12 CFZ doses, having better performance status, lower initial Hp level, higher hemoglobin, and lower creatinine at start of CFZ (Table 1). Direct antiglobulin test (DAT) was negative in all 10 tested patients, and ADAMTS13 levels were normal in all three tested patients with hemolysis. Glucose-6-phosphate dehydrogenase (G6PD) status was not tested, and supravital staining for Heinz bodies was not performed. Information on erythrocyte morphology in blood smears (hematoxylin and eosin stain) was available in 7 patients. No aberrations except mild anisocytosis were described in 6 patients. Schistocytes were present in the blood smear in only one patient (aged 57 years), who developed fulminant TMA with severe MAHA, profound thrombocytopenia, hypertension, and dialysis-dependent kidney failure 45 days after CFZ initiation (Figure 1). She eventually died from cerebral bleeding 6 days after TMA diagnosis, despite plasmapheresis initiation. Among remaining patients with hemolysis, 3 of 15 (20%) developed grade 1-2 elevations of creatinine during CFZ treatment. One patient had dialysis-dependent chronic kidney failure at CFZ treatment initiation. Mild elevations of liver transaminases (grade 1) were observed in 1 of 14 (7%) tested patients.

Two patients were retreated with CFZ after 2.5 and 3 years, respectively (hemolysis at the initial CFZ therapy line in both). One of them developed DAT-negative hemolytic anemia, requiring discontinuation of the retreatment. Hp returned to normal in another patient when CFZ doses were dispersed to every second week, but turned very low again when the ordinary schedule was restored. Maintenance with CFZ (one dose every second week) in two

TABLE 1 Patient and treatment characteristics, and their association with hemolysis

Parameter	Categories	No. (%)[a]	No. with hemolysis (%)[b]	HR (95% CI)	*P* value
Age at start of CFZ (y)	≤65	10 (42)	9 (90)	REF	.06
	>65	14 (58)	7 (50)	0.11 (0.01-1.13)	
Sex	Male	10 (42)	6 (60)	REF	.56
	Female	14 (58)	10 (71)	1.67 (0.3-9.27)	
WHO performance status	0-1	19 (79)	15 (79)	REF	**.03**
	2-3	5 (21)	1 (20)	**0.07 (0.01-0.77)**	
Diagnosis	Myeloma	20 (83)	14 (70)	REF	.45
	Other[c]	4 (17)	2 (50)	0.43 (0.05-3.79)	
Light chain	Lambda	14 (58)	8 (57)	REF	.25
	Kappa	10 (42)	8 (80)	3.0 (0.46-19.59)	
Paraprotein type	BJ only	6 (25)	3 (50)	REF	
	IgG	11 (46)	7 (64)	1.75 (0.23-13.16)	.59
	IgA	7 (29)	6 (86)	2.45 (0.65-9.23)	.19
Hemoglobin at start of CFZ (g/dL)	<10	7 (29)	2 (29)	REF	**.02**
	≥10	17 (71)	14 (82)	**11.67 (1.49-91.54)**	
Creatinine at start of CFZ (μmol/L)	>100	8 (33)	3 (37)	REF	**.04**
	≤100	16 (67)	13 (81)	**7.22 (1.08-48.47)**	
Haptoglobin at start of CFZ (g/L)	>1.9	6 (25)	1 (17)	REF	**.01**
	≤1.9	18 (75)	15 (83)	**25.0 (2.09-298.29)**	
No. of previous therapy lines	<3	9 (38)	5 (56)	REF	.37
	≥3	15 (62)	11 (73)	2.2 (0.38-12.57)	
Autologous SCT prior to CFZ	No	9 (38)	4 (44)	REF	.88
	Yes	15 (62)	12 (80)	5.0 (0.81-31.0)	
Time from diagnosis to CFZ (y)	<4	10 (42)	6 (60)	REF	.56
	≥4	14 (58)	10 (71)	1.67 (3.0-9.27)	
CFZ dose (mg/m^2)	27	5 (21)	3 (60)	REF	
	36	5 (21)	3 (60)	1.0 (0.08-12.56)	1.0
	56	14 (58)	10 (71)	1.67 (0.2-14.05)	.64
No. of CFZ doses	≤12	7 (29)	2 (29)	REF	**.02**
	>12	17 (71)	14 (82)	**11.67 (1.49-91.54)**	
Dexamethasone dose (mg)	4	4 (17)	2 (50)	REF	.45
	20	20 (83)	14 (70)	1.05 (0.92-1.21)	
Other concomitant therapy	No	19 (79)	12 (63)	REF	.49
	Yes[d]	5 (21)	4 (80)	2.33 (0.22-25.24)	

Values in bold indicate statistical significance.

Abbreviations: BJ, Bence Jones protein; CFZ, carfilzomib; CI, confidence interval; HR, hazard ratio, SCT, stem cell transplantation; WHO, World Health Organization.

[a]% of all patients

[b]% of patients in the category

[c]Light-chain (AL) amyloidosis (n = 3) and light-chain deposition disease (n = 1)

[d]Cyclophosphamide orally (n = 3) and thalidomide (n = 2)

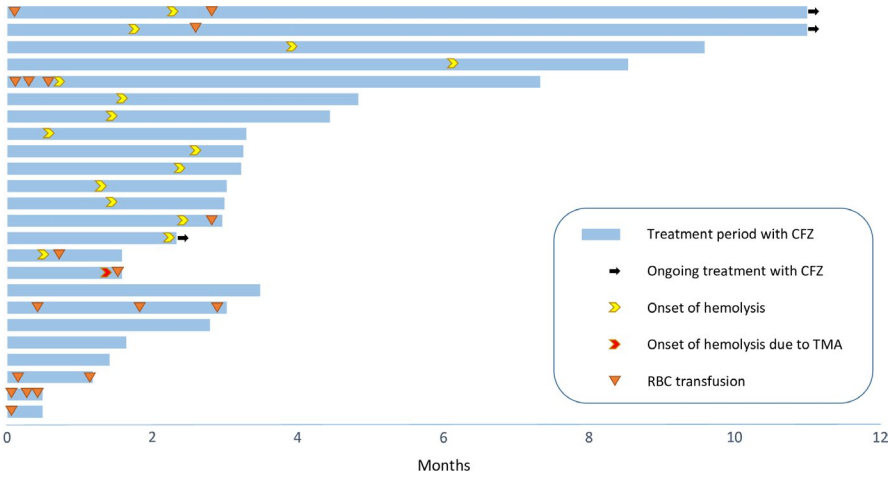

FIGURE 1 Swimmer plot graph demonstrating treatment duration with carfilzomib (CFZ) in 24 patients, with onset of hemolysis (haptoglobin <0.1 g/L) in 16 patients, as well as timing of red blood cell transfusions (RBC). Hemolysis due to thrombotic microangiopathy (TMA) was observed in one patient

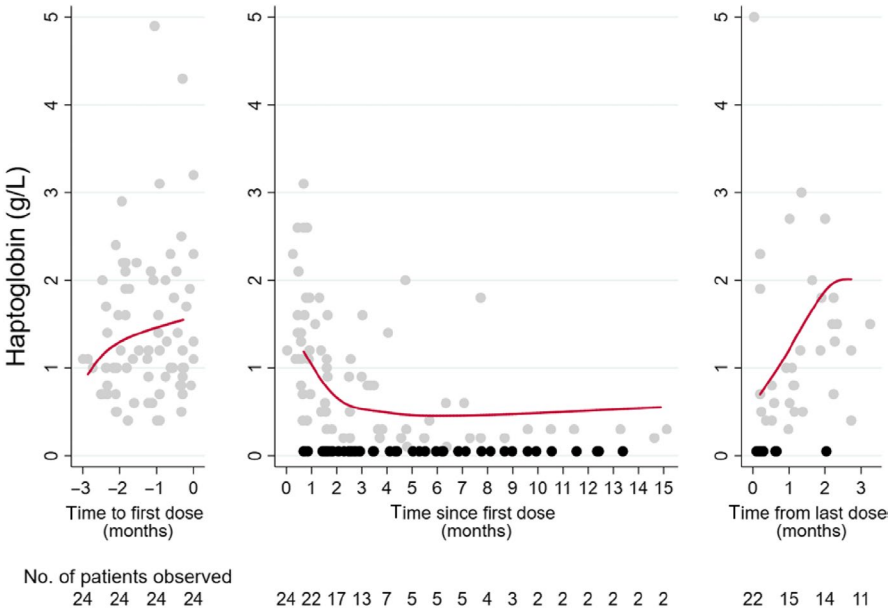

FIGURE 2 Trajectory plots of haptoglobin levels before, during, and after carfilzomib treatment. Gray dots represent observed single haptoglobin values. Black dots represent single haptoglobin values <0.1 g/L consistent with the presence of hemolysis. Red curves represent the average haptoglobin trajectories estimated by the best-fitting multilevel fractional polynomial model

patients, who previously had developed hemolysis on the ordinary CFZ schedule, did not result in low Hp. No signs of hemolysis were observed in our cohort from diagnosis until CFZ initiation, except during bortezomib treatment in 2 of 21 (9.5%) patients who each demonstrated a single value of Hp < 0.1 g/L, and in another patient on several occasions (unrelated to specific treatments).

4 | DISCUSSION

The high frequency of hemolysis, 67% in our unselected cohort of CFZ-treated patients, was striking. To our knowledge, only one recent study of high-dose CFZ in mostly first-line myeloma treatment has reported similar findings, with 8 of 10 patients showing modest hemolysis (but the biochemical markers were not specified).[12] In the present investigation, we used Hp levels to demonstrate associations of hemolysis with the time courses of CFZ therapy. We found no other explanations for low Hp, such as liver failure or malnutrition.[13] The hemolysis did not seem to be dose-dependent regarding single doses, but rather schedule-dependent, as it was not present with CFZ administration every second week. The incidence of hemolysis was higher when CFZ was given in more than 12 doses. Furthermore, it cannot be ruled out that hemolysis also was present in some patients with normal Hp during CFZ treatment. Inflammation, malignancy, and steroid usage can elevate Hp, possibly masking a hemolytic process.[13]

Assessing the pathogenesis of anemia during CFZ treatment in our cohort with heterogeneous, predominantly advanced and heavily pretreated disease is cumbersome. However, in most of our patients, the grade of hemolytic anemia seemed modest, as in the recent report.[12] This is in contrast to profound anemia in previously reported patients with CFZ-induced TMA.[7,9-11] Due to the absence of schistocytes (when assessed) and overt clinical signs of TMA in all except one of our patients, other hemolytic mechanisms should be considered. Autoimmune hemolysis was ruled out by negative DAT in all of the 10 tested patients. The ADAMTS13 level was normal in the three tested patients, including the one diagnosed with CFZ-induced TMA. The latter is in congruence with other reported

CFZ-induced TMA cases[4,6-11] and ruled out thrombotic thrombocytopenic purpura (TTP). G6PD deficiency is not prevalent in Scandinavia (<0.5%)[15] and was not considered as possible hemolysis mechanism in our cohort. Authors of the previously mentioned case series found normal levels of G6PD in all patients with CFZ-induced hemolysis.[12]

Oxidative stress as a trigger of mostly well-compensated hemolysis during CFZ treatment was proposed previously.[12] The retrospective nature of our study precluded collection of desirable additional laboratory data, including evaluation of the possible presence of Heinz bodies in the red blood cells (found by the recent report).[12] Here, we hypothesize that hemolysis may be induced by a previously described suppressive effect of CFZ on phosphorylation of eukaryotic translation initiation factor 2-alpha (eIF2α) by eIF2α kinase 1 (EIF2AK1), also known as heme-regulated inhibitor kinase (HRI).[16] Physiologically, intracellular heme depletion activates EIF2AK1, which shuts off protein synthesis and prevents excess of globin chains, protecting erythrocyte survival in states of iron deficiency.[17] Inhibition of EIF2AK1 by CFZ[15] may lead to accumulation of globin chains in erythrocytes, causing hemolysis.[17] Novel inhibitors of eIF2α dephosphorylation, with potential to both increase tumor cell death and attenuate hemolysis, might become available for combination therapy with proteasome inhibitors in the future.[18,19]

Both a recent report and our study thus suggest that modest hemolysis develops in a majority of CFZ-treated patients.[12] It is not clear whether CFZ-induced TMA is a further development from this hemolytic process or has a separate pathogenesis, where immune-mediated and dose-dependent toxic effects of CFZ have been proposed.[9] CFZ inhibition of vascular endothelial growth factor (VEGF) production could contribute to endothelial dysfunction and TMA.[7] The susceptibility to CFZ-induced TMA could depend on mutations in genes involved in complement activation, resulting in defective complement control. Heterozygous *CFHR3-CFHR1* deletions were found in two patients developing TMA during treatment with CFZ.[8] Innate susceptibility can be conditioned by ethnicity, as suggested by the occurrence of TMA in as many as 4 of 24 patients (17%) treated with CFZ in Singapore.[10] Different genetic profiles in terms of complement mutations have been reported in Chinese patients with TMA, compared with European and Japanese patients.[17] The hypothesis of complement pathway involvement in the pathogenesis of CFZ-induced TMA was supported by effectiveness of a complement monoclonal antibody, eculizumab, in four reported patients with this condition.[8,9,11] However, CFZ-induced TMA was self-limiting after drug termination in other cases.[5,7,9,10] Fatal outcomes (as in our patient) were also observed,[6,9] demonstrating the severity of this complication.

To conclude, our study confirmed in a larger setting that CFZ-treated patients with plasma cell disorders frequently develop hemolysis, by generally unclear mechanisms. Predisposing factors, pathogenic mechanisms, and clinical consequences deserve further investigation. Hemolysis parameters, platelet counts, and renal function tests should be assessed in patients presenting with anemia during CFZ therapy, with special vigilance regarding rare TMA development. Routine monitoring of Hp levels appears helpful for early identification of the condition. However, in the majority of CFZ-treated patients with laboratory signs of hemolysis, the clinical course appears benign and compatible with continued therapy, with recovery after treatment discontinuation. Reduced dosing frequency may be recommended for some affected patients.

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CONFLICT OF INTEREST

The authors have no affiliation with any organization with a direct or indirect financial interest in the subject matter discussed in the manuscript.

AUTHORS CONTRIBUTIONS

PK conceptualized and designed the study. PK, KKB, BU, and M.Å. contributed to data collection and interpretation. PK and KKB wrote the initial draft of the manuscript. PK, BU, and M.Å. reviewed and edited the manuscript. All authors approved the final version.

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