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Zonulin-related peptides in the setting of multiple myeloma — evaluation of a candidate molecule with respect to anemia, chronic kidney disease, and tumor burden: a pilot study

ABSTRACT

Introduction: The zonulin-related family of peptides is implicated in the regulation of intestinal permeability, inciting chronic inflammation and potentially in the incidence of cancer due to increased antigen trafficking. Scarce data are available on the potential role of serum zonulin-related peptides (ZRP) as biomarkers of hematologic cancer and related organ involvement.

Objectives: This study aimed to evaluate correlations between ZRP as assessed by the commercially — available Immunodiagnostik enzyme-linked immunosorbent assay (ELISA) assay, complete blood count, multiple myeloma (MM) stage, and renal impairment among MM patients.

Materials and methods: We analyzed a population of 73 patients with MM and evaluated the relationship between disease characteristics and long-term outcomes. The control group included 21 healthy volunteers (11 women, 10 men) between 24 and 69 years old. Serum ZRP were assayed using a commercially available kit (Immundiagnostik, Bergen, Germany).

Results: Twenty-six patients had eGFR < 60 mL/min/1.73 m^2. Median (IQR) serum concentration of ZRP in the studied group was 23.9 (19.9; 27.4) ng/mL. ZRP did not differ between subjects with and without anemia (defined as hemoglobin below the lower reference limit, $p = 0.4$). Significant correlations were detected with serum albumin ($R = 0.30$; $p = 0.009$), log (creatinine) ($R = –0.28$; $p = 0.018$), eGFR ($R = 0.26$; $p = 0.025$), ferritin ($R = 0.34$; $p = 0.013$), and log (NT-proBNP) ($R = –0.32$; $p = 0.006$). Moreover, in patients with symptomatic MM, ZRP correlated with monoclonal protein in serum ($R = –0.29$; $p = 0.046$), blood hemoglobin ($R = 0.27$; $p = 0.027$), and age ($R = –0.24$; $p = 0.044$). In multiple regression, serum concentrations of monoclonal protein and ferritin, as well as the International Staging System for multiple myeloma (ISS) stage 3, were identified as independent predictors of ZRP concentrations.

Conclusions: Serum concentrations of zonulin-related peptides only weakly correlate with kidney failure (creatinine and eGFR) in MM patients and anemia (hemoglobin concentration) in symptomatic MM patients. Serum ZRP assay is of little benefit in the setting of MM, exhibiting only weak correlations with indices of organ involvement, and it cannot be used to assess prognosis in MM.

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Key words: chronic kidney disease, multiple myeloma, zonulin-related peptides

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INTRODUCTION

Multiple myeloma (MM), a proliferative plasma cell dyscrasia, affects usually the elder population and is characterized by organ involvement: bone lesions, anemia, renal insufficiency, and hypercalcemia [1]. The most frequent manifestation of MM is anemia which can occur in up to 73% of all patients while kidney failure appears in up to 48%. Cytokines produced by plasmacytes lead to anemia of chronic disease (ACD), by erythropoiesis inhibition and impaired iron homeostasis [2]. Renal failure should also be taken into consideration as an important factor leading to anemia in MM patients. It is well known that decreased renal function and advanced anemia lead to severe complications in MM and poor prognosis. The incidence rate of MM is estimated at up to 2.1 per 100 000 individuals. [3] Moreover, Liu et al. showed that severe anemia is an independent risk factor for renal impairment in MM [4]. Nevertheless, anemia is part of the clinical picture in both MM and chronic kidney disease (CKD). This is the reason to search for some specific biomarkers of anemia that may not be confounded by renal impairment in MM patients.

Zonulin is a multifunctional protein that is tied to the haptoglobin family of acute-phase proteins, which play an important role in hemoglobin scavenging. Initially, zonulin was perceived as an inactive precursor of haptoglobin 2. Recent studies have shown that the intact single chain may regulate intestinal permeability via endothelial growth factor receptor (EGFR) transactivation, while the two-chain form of the protein, resulting from the cleavage of the single chain has the putative function of a hemoglobin scavenger. Over time, haptoglobins have changed from complement-associated proteins to molecules with the ability to modulate intercellular tight junctions. Researchers have hypothesized that increased membrane permeability (i.e., tied to the zonulin pathway) is an innate defense mechanism against microorganisms. It has been further proposed that a genetic predisposition, coupled with gut permeability (i.e., the increased exposure to environmental antigens) and immune dysfunction, may lead to chronic inflammatory diseases, such as colitis, arthritis, cardiovascular and chronic kidney diseases [5–12].

Studies have shown that zonulin is low when renal function is impaired [13]. An association with metabolic indices (e.g. glucose, lipids) [14–16] has been described in some studies [13], while the relationship with inflammation is unclear [11]. Other reports based on enzyme-linked immunosorbent assay (ELISA) have suggested that zonulin levels do not differ between patients with chronic infection, cancer, and control subjects [12]. However, the discrepancies across studies may stem from the limitations in laboratory methods of zonulin assessment. Recent reports have shown that the immunodiagnostic assay may actually detect a number of zonulin-related proteins, which falls in line with the hypothesis that there is a zonulin protein family (however, whether functional similarities are present remains unclear) [8, 17]. Researchers have described that properdin, which is the only positive regulator of complement proteins, is the molecule that is identified by the Immundiagnostik ELISA assay [17, 18].

This study aimed to analyze zonulin-related peptides (ZRP) as a marker of anemia (correlation with red blood cell count, hemoglobin, hematocrit, and red blood cell indices), iron status markers (including ferritin), and the relationship between ZRP and acclaimed parameters of renal failure (creatinine, estimated glomerular filtration rate — eGFR), stages of MM, and prognosis for MM patients.

MATERIALS AND METHODS

STUDY DESIGN AND PATIENTS

Patient recruitment was performed during ambulatory control visits at the Department of Haematology of the University Hospital in Kraków, Poland. The inclusion criteria, according to the International Myeloma Working Group, were age ≥ 18 years and the diagnosis of smoldering myeloma (SMM) or MM. In contrast, recent active infection, history of hepatitis B, C, HIV, and neoplasms other than myeloma were exclusion criteria. Detailed histories of the disease from all patients were collected from available medical records. Data on age, sex, the date of initial diagnosis of SMM or MM, current diagnosis, presence of bone lesions in X-ray, and information about past and present treatment including response to treatment (complete response [CR], partial response [PR], stable disease [SD], progressive disease [PD]) were collected at the initial study visit. Data on mortality were assembled during follow-up. The median observation time was 19 months.

The control group included 21 healthy volunteers (11 women, 10 men) between 24 and 69 years old. These subjects were recruited solely to provide blood samples that were used to obtain control results of non-standard laboratory tests.

The study was conducted according to the principles of the Declaration of Helsinki and in compliance with the International Conference on Harmonization Good Clinical Practice regulations. The study was approved by the Bioethics Committee of the Jagiellonian University (approval number 1072.6120.248.2017), and all patients signed informed consent for participation in the study.

BLOOD SAMPLES AND LABORATORY TESTS

On the day of blood collection, in the morning following overnight fasting and rest, routine laboratory tests of MM patients were performed and included complete blood counts, serum concentrations of creatinine, serum activity of lactate dehydrogenase, total protein, albumin, β2-microglobulin, free light chains, urine concentrations of light chains, alanine and aspartate aminotransferases, and ferritin.

Serum samples for other laboratory tests were aliquoted and stored at a temperature below –70°C. These non-routine laboratory tests included N-terminal prohormone of brain natriuretic peptide (NT-proBNP), ZRP, hepcidin, sTfR, GDF-15, and interleukin 6 (IL-6).

Automatic biochemical analyzers: Hitachi 917 (Hitachi, Japan) and Modular P (Roche Diagnostics, Mannheim, Germany) were used. Hematological parameters were measured using Sysmex XE 2100 analyzer (Sysmex, Kobe, Japan). The concentration of serum free light chains (FLC), urine LC (κ and λ type), and β2-microglobulin were measured by the immunonephelometric method on a BN II analyzer (Siemens GmbH, Germany). The determination of free light chains (FLC κ, FLC λ) was performed using Freelite reagents (Binding Site, Birmingham, UK) with reference ranges: 1.7–3.7 g/L and 0.9–2.1 g/L, respectively. The immunophenotype of monoclonal protein was determined by serum immunofixation (IFE) on agarose gel (EasyFix G26, Interlab, Italy).

The eGFR was calculated based on serum creatinine using the 2009 Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) formula [GFR (mL/min/1.73 m^2) = 141 × min (serum creatinine/k, 1) α × max (serum creatinine/k, 1) – 1.209 × 0.993 age × 1.018 (if female) × 1.159 (if black); where k is 0.7 for females and 0.9 for males. A is –0.329 for females and –0.411 for males, min indicates minimum serum creatinine/k or 1, and max indicates maximum serum creatinine/k or 1] based on serum creatinine.

The non-routine laboratory tests were performed in series, using commercially available immunoenzymatic test kits. Serum IL-6 was measured using Quantikine ELISA Human IL-6 Immunoassay (R&D Systems, Inc., Minneapolis, USA), with the minimum detectable dose of 0.70 pg/mL, and the intra- and interassay precision of 2.0% and 3.8%, respectively. The reference range for IL-6 was 3.13–12.5 pg/mL. NT-pBNP concentrations in serum were measured by the Enzyme-linked Immunosorbent Assay Kit For NT-ProBNP Human (Cloud-Clone Corporation, Huston, TX, USA), with the minimum detectable dose of 11.7 pg/mL, the quantification range of 30.9–2500 pg/mL and the intra- and interassay precision of 10% and 12%, respectively. Serum ZRP were measured using IDK Zonulin ELISA (Immundiagnostik AG, Bensheim, Germany), with the minimum detectable dose ranging from 3.03 to 40.25 ng/ml; limit of detection of 0.183 ng/mL and the intra- and interassay precision of 3.5% and 8.3%, respectively. Serum hepcidin 25 levels were measured using Hepcidin 25 human Cet. No. S-1337 kit (Peninsula Laboratories International, Inc., San Carlos, CA, USA). The reference range for hepcidin 25 was 0.02–25 pg/mL. Serum soluble transferrin receptor (sTfR) was measured using Quantikine IVD ELISA Human sTfR Immunoassay (R&D Systems, Inc., Minneapolis, MN, USA), with the minimum detectable concentration of 0.5 nmol/L, and the intra- and interassay precision of 6.2% and 5.7%, respectively. Serum GDF-15 was measured using Quantikine ELISA Human GDF-15 Immunoassay (R&D Systems, Inc., Minneapolis, USA), with the minimum detectable concentration ranging from 0.0 to 4.4 pg/mL, and the intra- and interassay precision of 2.8% and 5.6%, respectively.

STATISTICAL ANALYSIS

Data were reported as number of patients and percentage of the group for categories. Median, lower quartile (Q1), and upper quartile (Q3) were reported for quantitative variables since most variables' distributions differed from normal. The Shapiro-Wilk test was used to assess normality. The associations between ZRP and other studied laboratory markers

Table 1. Baseline clinical characteristics of studied patients with MM (n=73).

Characteristic	Values
Median age (Q1; Q3), years	70 (62–76)
Female sex, n (%)	35 (48)
Median time since diagnosis (Q1; Q3), months	36 (17; 69)
Smoldering MM, n (%)	6 (8)
ISS* stage I, n (%) stage II, n (%) stage III, n (%)	 40 (55) 15 (21) 12 (16)
Immunofixation IgG, n (%) IgA, n (%) IgM, n (%) κ, n (%) λ, n (%) biclonal, n (%) non-secretory MM, n (%)	 52 (71) 17 (23) 1 (1) 45 (62) 26 (36) 2 (3) 3 (4)
Disease state CR, n (%) PR, n (%) SD, n (%) PD, n (%)	 22 (30) 30 (41) 6 (8) 15 (21)
Number of prior treatment schemes no treatment, n (%) 1, n (%) 2 or more, n (%)	 8 (11) 17 (23) 48 (66)
Maintenance treatment, n (%)	30 (41)
History of auto-PBSCT, n (%)	28 (38)
Bone lesions, n (%)	44 (60)
Anemia, n (%)	14 (19)
eGFR < 60 mL/min/1.73 m^2	23 (32)

*International Staging System for multiple myeloma: stage I serum: β2-microglobulin < 3.5 mg/L and serum albumin ≥ 35 g/L; stage II: β2-microglobulin < 3.5 mg/L and serum albumin < 35 g/L or β2--microglobulin 3.5–5.5 mg/L and serum albumin independently; stage III: β2-microglobulin > 5.5 mg/L and serum independently. CR — complete response; eGFR — estimated glomerular filtration rate; ISS — International Staging System for multiple myeloma; MM — multiple myeloma; PBSCT — peripheral blood stem cell transplant; PD — progressive disease; PR — partial response; SD — stable disease

at the start of the study and at the end of the follow-up were determined by the Pearson coefficient and multiple regression analysis; right-skewed variables were log-transformed before inclusion in the analyses. Forward stepwise regression was used to search for the variables independently associated with serum ZRP at baseline. All the variables that significantly correlated with serum ZRP in simple analysis (at $p < 0.05$) were included in the forward stepwise regression analysis. The baseline and follow-up values were compared using paired t-test or Wilcoxon matched pairs test, according to distribution. Simple Cox proportional hazard regression was used to verify the relationship between baseline zonulin and overall mortality. The survival time was calculated from initial patient recruitment until death or last follow-up. The statistical tests were two-tailed and $p < 0.05$ indicated statistical significance. Statistica 12.0 (StatSoft, Tulsa, OK, USA) was used for computations.

RESULTS

CHARACTERISTICS OF THE STUDIED GROUP

The study included 73 patients with MM (67 symptomatic, 6 smoldering), 35 women and 38 men, with median age 70 (Q1; Q3: 62–76) years. Median (Q1; Q3) time from initial MM diagnosis was 36 (17; 69) months. According to the International Staging System for multiple myeloma (ISS), 40 patients were in ISS stage I, 15 in stage II, and 12 in stage III at the time of blood collection. Remission of MM was diagnosed in 52 patients, and stable or progressive disease in 21. Twenty-three patients had eGFR < 60 mL/min/1.73 m^2 (Tab. 1).

Average blood hemoglobin was close to the lower reference limit (Tab. 2) although anemia (defined as blood hemoglobin below the lower reference limit of 11 g/dL in women and 12 g/dL in men) was diagnosed in 14 (19%) patients.

VARIABLES ASSOCIATED WITH SERUM ZONULIN-RELATED PEPTIDES AT BASELINE

Median (Q1; Q3) serum ZRP in the studied group was 23.87 (19.93; 27.44) ng/mL (Tab. 2). These values were significantly lower as compared to those observed in healthy individuals ($p < 0.001$; Fig. 1). Moreover, MM patients were characterized by higher IL-6, GDF-15, and sTfR than control subjects: median (Q1; Q3) in the control group were 0.58 (0.19; 1.13) pg/mL ($p < 0.001$); 584.00 (516.00; 762.00) pg/mL ($p < 0.001$); and 19.45 (17.28; 23.69) nmol/L ($p = 0.001$), respectively. Serum hepcidin in the control group [27.10 (19.97; 37.27) ng/mL] was similar to that in MM patients ($p = 0.9$). There were no differences in serum ZRP concentrations between patients with smoldering versus symptomatic MM, with ISS I to III ($p = 0.7$), with remission vs stable or progressive MM ($p = 0.9$), or with eGFR < 60 mL/min/1.73 m^2 versus those with higher eGFR ($p = 0.6$). Also, ZRP did not differ between subjects with and without anemia ($p = 0.4$) (Fig. 1). In patients with symptomatic MM, serum ZRP concentration was weekly correlated with age ($R = –0.25$; $p = 0.044$).

Table 2. Laboratory results at baseline in studied patients with MM (n = 73). Data are shown as median (Q1; Q3)

Laboratory test	Results
Leukocyte count, × $10^3/\mu L$	6.07 (4.89; 6.94)
Red blood cell count, × $10^6/\mu L$	4.04 (3.75; 4.44)
Hemoglobin, g/dL	12.80 (11.70; 13.60)
Hematocrit, %	37.30 (34.70; 39.30)
MCV, fl	90.80 (87.90; 94.70)
MCH, pg	31.40 (30.00; 32.20)
MCHC, g/dL	34.40 (33.50; 34.80)
RDW-CV, %	14.30 (13.70; 15.30)
Platelet count, × $10^3/\mu L$	169.00 (141.00; 197.00)
Serum creatinine, μmol/L	89.00 (76.00; 104.00)
eGFR, mL/min/1.73 m^2	66.92 (50.29; 78.13)
Albumin, g/L	42.00 (39.80; 45.00)
2-microglobulin, mg/L	2.75 (2.17; 4.20)
Monoclonal protein, g/L	4.57 (2.19; 8.61)
Involved serum FLC, mg/L	38.30 (17.40; 106.00)
Lactate dehydrogenase, U/L	352.00 (308.00; 392.00)
Bilirubin, μmol/L	7.19 (5.60; 10.20)
Alanine aminotransferase, U/L	21.00 (16.00; 29.00)
Aspartate aminotransferase, U/L	22.00 (17.00; 27.00)
Interleukin 6, pg/mL	2.97 (1.61; 6.00)
Fibrinogen, g/L	3.70 (3.00; 4.80)
Ferritin, μg/L*	166.00 (64.00; 380.00)
Hepcidin, ng/mL	28.83 (16.54; 44.63)
sTfR, nmol/L	25.02 (20.45; 28.37)
GDF-15, pg/mL	1259.00 (863.20; 1934.00)
Zonulin-related peptides, ng/mL	23.87 (19.93; 27.44)
NT-proBNP, pg/mL	74.80 (31.57; 287.20)

*Available in 51 patients; eGFR — estimated glomerular filtration rate; FLC — free light chains; GDF-15 — growth differentiation factor 15; MCH — mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV — mean cell volume; NT-proBNP — N-terminal pro-B-type natriuretic peptide; RDW-CV — red cell distribution width — coefficient of variation; sTfR — soluble transferrin receptor

Serum ZRP concentration was not associated with the time since MM diagnosis.

In the whole studied group, significant correlations were observed between ZRP and serum albumin, log (creatinine), eGFR, log (bilirubin), log (ferritin), log (NT-proBNP), and MCHC (Tab. 3). Moreover, in patients with symptomatic MM, ZRP correlated with monoclonal protein in serum, blood hemoglobin, and log (GDF-15) (Tab. 3). Other studied laboratory markers were not significantly associated with serum ZRP.

We applied multiple linear regression to examine which variables are independently associated with serum ZRP concentrations in studied patients. The variables, listed in Table 3, were included in a forward stepwise multiple linear regression analysis together with age and symptomatic (vs smoldering) MM. In the resulting regression model presented in Table 4, serum concentrations of monoclonal protein and log (ferritin) were identified as independent statistically significant predictors of serum ZRP concentrations (Tab. 3).

THE ASSOCIATION BETWEEN BASELINE SERUM ZONULIN-RELATED PEPTIDES AND FOLLOW-UP DATA

We analyzed associations between baseline serum zonulin-related peptides and serum creatinine, eGFR, and complete blood count at the end of follow-up in the MM patient group. These follow-up data were available for all MM patients: in the case of patients who died, their last available data were used. There were 15 deaths (21% of the studied group): six patients died due to MM, five due to infection, and three due to other neoplasms; the cause of one death was unknown. The median observation time was 19 months (Q1–Q3: 15-22 months). Baseline ZRP concentration was not associated with mortality in simple Cox regression (hazard ratio: 1.01; 95% confidence interval: 0.93–1.10; p = 0.8). At the end of observation, median eGFR [64.90; (47.77; 79.26) mL/min/1.73 m^2; p = 0.013] and median blood hemoglobin [11.90; (15.90; 13.40) g/dL; p = 0.019] were slightly lower compared to the initial values [66.92 (50.29; 78.13) mL/min/1.73 m^2 and 12.80 (11.70; 13.60) g/dL, respectively]. Baseline serum ZRP did not predict final values of eGFR, red blood count, hemoglobin, or hematocrit nor changes in the results of these tests during the follow-up. The only significant correlation was found between baseline ZRP and final mean cell hemoglobin concentration (MCHC) (R = 0.25; p = 0.035 in the whole studied group and R = 0.31; p = 0.011 in patients with symptomatic MM).

DISCUSSION

To our knowledge (after searching Pubmed and GoogleScholar on 11.01.2022), data on the family of zonulin-related peptides in MM patients have not been published. Little data are available on serum zonulin assessments in patients with malignancy despite reports that it may be related to a variety of cancers [19]. In previous studies with cancer patients, zonulin levels were not significantly

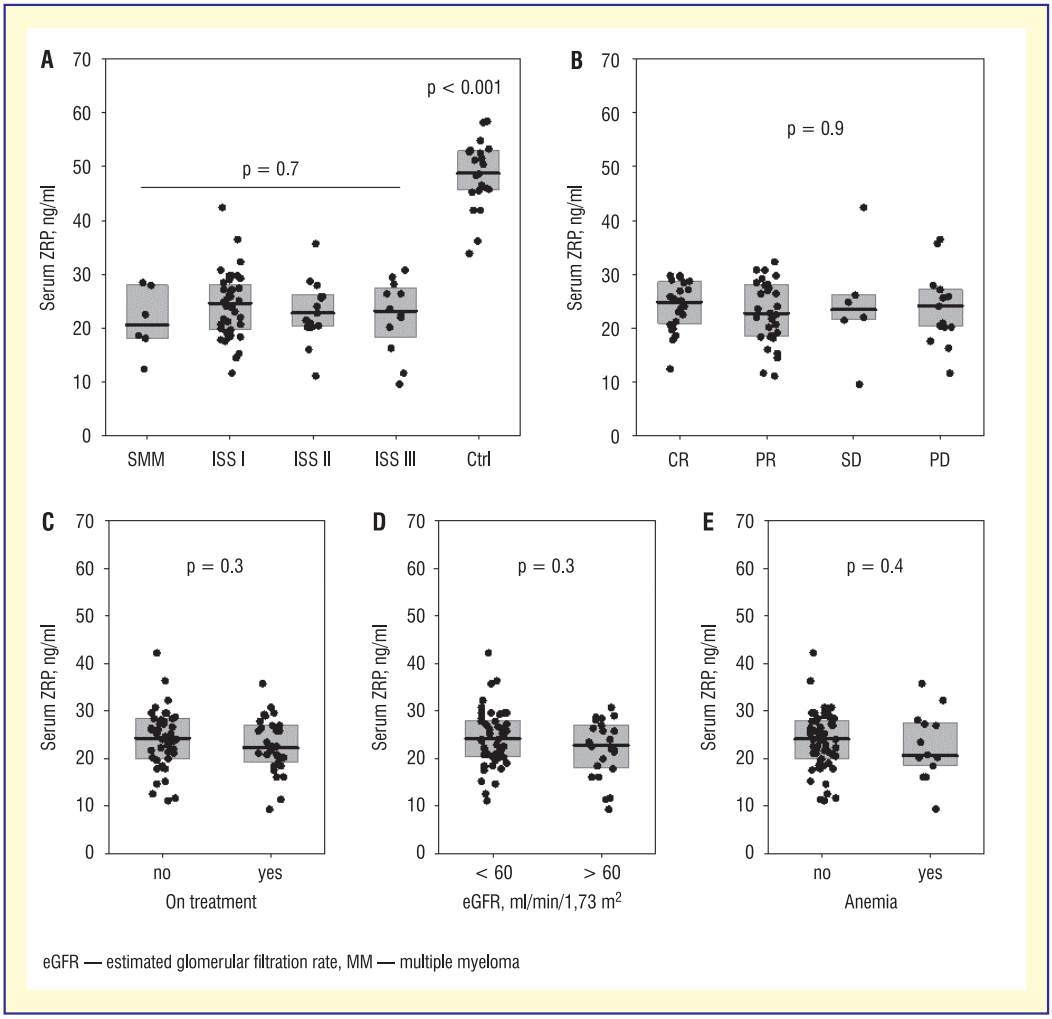

Figure 1. Serum zonulin-related peptides (ZRP) among MM patients with increasing MM stage in comparison to healthy controls (**A**); serum ZRP in MM patients according to response to treatment (**B**), maintenance treatment status (**C**), eGFR (**D**), and anemia (**E**). Data are shown as median (central line), interquartile range (box), and raw data (points)

Table 3. Simple correlations between serum zonulin-related peptides and other studied laboratory markers at baseline

Variable	All patients (n = 73)		Symptomatic MM (n = 67)	
	R	p	R	p
Albumin	0.30	0.009	0.33	0.006
log (serum creatinine)	–0.28	0.018	–0.29	0.016
eGFR	0.26	0.025	0.30	0.013
Hemoglobin	–0.19	0.112[NS]	0.27	0.027
MCHC	0.29	0.014	0.32	0.008
log (ferritin)*	0.28	0.045	0.31	0.034
log (bilirubin)	0.27	0.026	0.33	0.009
Serum monoclonal protein	–0.25	0.068[NS]	–0.29	0.046
log (NT-proBNP)	–0.32	0.006	–0.41	0.001
log (GDF-15)	–0.21	0.074[NS]	–0.27	0.030

*Available in 51 patients; [NS]Non-significant result; eGFR — estimated glomerular filtration rate; GDF-15 — growth differentiation factor 15; MCHC — mean cell hemoglobin concentration; MM — multiple myeloma; NT-proBNP — N-terminal pro-B-type natriuretic peptide

Table 4. Multiple linear regression model to predict serum concentrations of zonulin-related peptides in the studied group of 73 patients with MM

Independent variable	Standardized regression coefficient ± standard error	p
Serum monoclonal protein	–0.35 ± 0.15	0.025
log (ferritin)	0.31 ± 0.15	0.039
log (serum creatinine)	–0.31 ± 0.16	0.060^{NS}
log (NT-proBNP)	–0.19 ± 0.16	0.209^{NS}
R^2 for the model	0.32	0.010

NT-proBNP — N-terminal pro-B-type natriuretic peptide.

different from control populations [12, 20]. Some researchers have proposed that increased antigen trafficking through the gut may be a component of the immune imbalance culminating in cancer onset [6]. However, based on the available knowledge, and the shortcomings of current testing technology, these hypotheses remain speculative in nature.

Circulating zonulin-related peptides may be high, low, or on a similar level to healthy controls when comparing patients with different types of cancer. In the present study, ZRP levels were significantly lower than in reference subjects but did not differ across progressive disease patients. In studies of breast cancer patients examining the impact of cytotoxic agents on indices of mucosal permeability, zonulin levels did not significantly change over time despite follow-up during the chemotherapeutic protocol [20]. Serum zonulin has been shown to be significantly elevated in patients with hepatocellular carcinoma, as well as late-stage liver cirrhosis due to chronic viral infection [21]. Studies have described a relationship between zonulin and markers of systemic inflammation (tumor necrosis factor-alpha and IL-6) [22], which falls in line with a significant positive relationship between septicemia and zonulin that has been described in the context of postoperative complications [23]. The list of chronic inflammatory diseases in which zonulin is investigated as an indicator of intestinal permeability is extensive and encompasses metabolic and immune imbalance [6]. The relationship between indices of metabolic disease (e.g., diabetes, hypertension, obesity) and increased serum zonulin concentrations is frequently reported and represents another potential confounder [15, 16, 24]. Finally, zonulin is increased in elderly persons without any apparent pathology and is inversely tied to indicators of frailty [22]. These observations show that the interpretation of zonulin-related protein assay is very difficult in cancer patients as it may be interfaced with a variety of immune or metabolic processes that occur outside the context of malignancy. In-depth clarification of the biological role of zonulin-related proteins is necessary to reach a more definite conclusion regarding the utility of this novel biomarker assay.

In the total group of myeloma patients, we observed significant correlations between ZRP and serum albumin, parameters of kidney function, hemoglobin breakdown, and cardiac overload. In prior studies, serum zonulin has been shown to be lower in patients with chronic kidney disease. No relationship between zonulin and the presence of an inflammatory state (defined as highly sensitive C-reactive protein > 10 mg/dL) was previously noted for patients in the early stages of renal disease [11]. Furthermore, an association with iron-regulatory hepcidin was observed only in kidney-disease patients with higher pro-inflammatory markers.

The interpretation of the relationships described in the present study is unclear, as our sample is highly heterogeneous, at different stages of treatment, and the observed correlations are weak to modest. The relationship between serum ZRP assay with indicators of tumor burden, as well as kidney and cardiac function was explored in a multiple linear regression model and analyzed in the context of long-term follow-up. Although ferritin and paraprotein levels are independent predictors of serum ZRP, the significance of this finding is complicated. In organ transplant recipients, zonulin was not associated with iron parameters [11, 25, 26]. In these studies, the authors hypothesized that lower zonulin levels might be observed due to impaired immunity following immunosuppressive regimens [25, 26]. Despite a structural relationship between zonulin-related proteins and iron-scavenging haptoglobin 2, there is little evidence to suggest a relationship with renal failure, anemia, iron metabolism, or immunosuppressive medication in MM patients.

In this study, we analyzed the association between ZRP and hematologic cancer on the example of MM, which is a new topic for researchers. We showed that in this type of neoplasm, there is little benefit of the serum ZRP assay as a tool to predict organ involvement.

In the present study, we observed that the detected ZRP were significantly lower in MM patients, as compared with healthy persons. It should be noted that researchers have described that the ELISA kit for ZRP (Immundiagnostik, Bensheim, Germany) may target a group of related molecules that are connected structurally, but not necessarily functionally [17]. We hypothesize that our findings may be due to either malignant, inflammatory, or metabolic components that lead to shifts in the circulating concentration of ZRP and account for the differences in healthy individuals.

Although structurally similar to haptoglobin 2 and implicated in the regulation of intestinal permeability (which may, in turn, affect immune dysfunction due to antigen exposure). In addition, a purported relationship in hemoglobin scavenging is reported. Zonulin concentrations are not only subject to alterations in a variety of inflammatory and metabolic diseases but are significantly changed in healthy aging. There are considerable limitations of testing technology, in which zonulin may not be the main detected peptide but rather one of the structurally similar molecules (of unknown functional relationship).

Properdin, a ~50 kDa glycoprotein, which is described as the only known positive regulatory mediator of complement, has been previously identified as the molecule likely detected by the zonulin assay [17, 18]. Complement is variably activated or dysregulated in a variety of malignancies but can also be expressed in the tumor microenvironment. Infiltrating immune cells are the purported source of properdin in the disease microenvironment, which may indicate a potential prognostic role for increased survival [18]. The notion of properdin being detected as the major component of ZRP by the immunodiagnostic assay is a potential explanation for the independent relationship between monoclonal protein and ferritin (i.e., an acute phase reactant and indicator of inflammation). However, until the exact targets of the assay are characterized, and the functional relationship of ZRP is clarified, the interpretation of findings remains highly speculative. Zonulin-related peptides detected in serum did not meaningfully predict changes in kidney function or anemia parameters in follow-up, which suggests that these molecules are unlikely candidates to monitor processes of organ involvement in MM.

Several limitations of our study have to be acknowledged. The study group involved a heterogeneous patient population subjected to different treatment modalities; therefore, clinical differences associated with varying stages of progression and organ manifestations may occlude the relationship between the investigated processes. We attempted to account for these issues by appropriate adjustments in multiple models. Another weakness is the lack of prospective appraisal of changes in the studied biomarkers over time; this limitation was caused by financial constraints. Although the findings should be interpreted with caution, this study has several strengths: this is the first study that has looked into the association between zonulin and hematologic cancer on the example of MM, and the data showed that despite the purported relationships with chronic inflammation and tumor burden, the serum zonulin-related peptides assay is of little benefit in the setting of MM, exhibiting only weak correlations with indices of organ involvement.

CONCLUSIONS

Anemia is often present at diagnosis of MM, in many cases as the first symptom of the disease. Our results suggest that zonulin-related peptides are not good predictors of anemia in MM patients. ZRP correlated with renal impairment, age, and malnutrition in MM patients, but it cannot predict progression of chronic kidney disease or anemia. Further development of testing technologies and elucidation of the mechanistic role of ZRP in malignancy is warranted.

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CONFLICT OF INTEREST

None declared.

References

1. Rajkumar S, Dimopoulos M, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. The Lancet Oncology. 2014; 15(12): e538–e548, doi: 10.1016/s1470-2045(14)70442-5, indexed in Pubmed: 25439696.
2. Ludwig H, Pohl G, Osterborg A. Anemia in multiple myeloma. Clin Adv Hematol Oncol. 2004; 2: 233–241, indexed in Pubmed: 16163188.
3. Cowan AJ, Allen C, Barac A, et al. Global Burden of Multiple Myeloma: a systematic analysis for the global burden of disease study 2016. JAMA Oncol. 2018; 4(9): 1221–1227, doi: 10.1001/jamaoncol.2018.2128, indexed in Pubmed: 29800065.
4. Liu MM, Xu HZ, Feng XM, et al. [Clinical research about risk factors and reversible predictors in renal impairment due to multiple myeloma]. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2015; 23(3): 722–727, doi: 10.7534/j.issn.1009-2137.2015.03.024, indexed in Pubmed: 26117025.
5. Ajamian M, Steer D, Rosella G, et al. Serum zonulin as a marker of intestinal mucosal barrier function: May not be what it seems. PLoS One. 2019; 14(1): e0210728, doi: 10.1371/journal.pone.0210728, indexed in Pubmed: 30640940.
6. Fasano A. All disease begins in the (leaky) gut: role of zonulin-mediated gut permeability in the pathogenesis of some chronic inflammatory diseases. F1000Res. 2020; 9, doi: 10.12688/f1000research.20510.1, indexed in Pubmed: 32051759.
7. Fasano A. Zonulin, regulation of tight junctions, and autoimmune diseases. Ann N Y Acad Sci. 2012; 1258: 25–33, doi: 10.1111/j.1749-6632.2012.06538.x, indexed in Pubmed: 22731712.
8. Fasano A. Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. Ann N Y Acad Sci. 2000; 915: 214–222, doi: 10.1111/j.1749-6632.2000.tb05244.x, indexed in Pubmed: 11193578.
9. Fasano A, Not T, Wang W, et al. Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. The Lancet. 2000; 355(9214): 1518–1519, doi: 10.1016/s0140-6736(00)02169-3, indexed in Pubmed: 10801176.
10. Leech B, McIntyre E, Steel A, et al. Risk factors associated with intestinal permeability in an adult population: A systematic review. Int J Clin Pract. 2019; 73(10): e13385, doi: 10.1111/ijcp.13385, indexed in Pubmed: 31243854.
11. Lukaszyk E, Lukaszyk M, Koc-Zorawska E, et al. Zonulin, inflammation and iron status in patients with early stages of chronic kidney disease. Int Urol Nephrol. 2018; 50(1): 121–125, doi: 10.1007/s11255-017-1741-5, indexed in Pubmed: 29134616.
12. Tajik N, Frech M, Schulz O, et al. Targeting zonulin and intestinal epithelial barrier function to prevent onset of arthritis. Nat Commun. 2020; 11(1): 1995, doi: 10.1038/s41467-020-15831-7, indexed in Pubmed: 32332732.
13. Carpes LS, Nicoletto BB, Canani LH, et al. Could serum zonulin be an intestinal permeability marker in diabetes kidney disease? PLoS One. 2021; 16(6): e0253501, doi: 10.1371/journal.pone.0253501, indexed in Pubmed: 34170951.
14. Moreno-Navarrete JM, Sabater M, Ortega F, et al. Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance. PLoS One. 2012; 7(5): e37160, doi: 10.1371/journal.pone.0037160, indexed in Pubmed: 22629362.
15. Zak-Gołąb A, Kocełak P, Aptekorz M, et al. Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects. Int J Endocrinol. 2013; 2013: 674106, doi: 10.1155/2013/674106, indexed in Pubmed: 23970898.
16. Zhang D, Zhang L, Zheng Y, et al. Circulating zonulin levels in newly diagnosed Chinese type 2 diabetes patients. Diabetes Res Clin Pract. 2014; 106(2): 312–318, doi: 10.1016/j.diabres.2014.08.017, indexed in Pubmed: 25238913.
17. Scheffler L, Crane A, Heyne H, et al. Widely used commercial ELISA does not detect precursor of haptoglobin2, but recognizes properdin as a potential second member of the zonulin family. Front Endocrinol (Lausanne). 2018; 9: 22, doi: 10.3389/fendo.2018.00022, indexed in Pubmed: 29459849.
18. Mangogna A, Varghese PM, Agostinis C, et al. Prognostic value of complement properdin in cancer. Front Immunol. 2020; 11: 614980, doi: 10.3389/fimmu.2020.614980, indexed in Pubmed: 33542722.
19. Fasano A. Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiol Rev. 2011; 91(1): 151–175, doi: 10.1152/physrev.00003.2008, indexed in Pubmed: 21248165.
20. Russo F, Linsalata M, Clemente C, et al. The effects of fluorouracil, epirubicin, and cyclophosphamide (FEC60) on the intestinal barrier function and gut peptides in breast cancer patients: an observational study. BMC Cancer. 2013; 13: 56, doi: 10.1186/1471-2407-13-56, indexed in Pubmed: 23379680.
21. Wang X, Li MM, Niu Ye, et al. Serum zonulin in HBV-associated chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma. Dis Markers. 2019; 2019: 5945721, doi: 10.1155/2019/5945721, indexed in Pubmed: 31485278.
22. Qi Y, Goel R, Kim S, et al. Intestinal permeability biomarker zonulin is elevated in healthy aging. J Am Med Dir Assoc. 2017; 18(9): 810.e1–810.e4, doi: 10.1016/j.jamda.2017.05.018, indexed in Pubmed: 28676292.
23. Liu Z, Li C, Huang M, et al. Positive regulatory effects of perioperative probiotic treatment on postoperative liver complications after colorectal liver metastases surgery: a double-center and double-blind randomized clinical trial. BMC Gastroenterol. 2015; 15: 34, doi: 10.1186/s12876-015-0260-z, indexed in Pubmed: 25881090.
24. Ohlsson B, Orho-Melander M, Nilsson PM. Higher levels of serum zonulin may rather be associated with increased risk of obesity and hyperlipidemia, than with gastrointestinal symptoms or disease manifestations. Int J Mol Sci. 2017; 18(3), doi: 10.3390/ijms18030582, indexed in Pubmed: 28282855.
25. Malyszko J, Koc-Zorawska E, Levin-Iaina N, et al. Zonulin, iron status, and anemia in kidney transplant recipients: are they related? Transplant Proc. 2014; 46(8): 2644–2646, doi: 10.1016/j.transproceed.2014.09.018, indexed in Pubmed: 25380885.
26. Przybyłowski P, Nowak E, Janik L, et al. Zonulin and iron metabolism in heart transplant recipients. Transplant Proc. 2014; 46(8): 2856–2859, doi: 10.1016/j.transproceed.2014.09.041, indexed in Pubmed: 25380935.