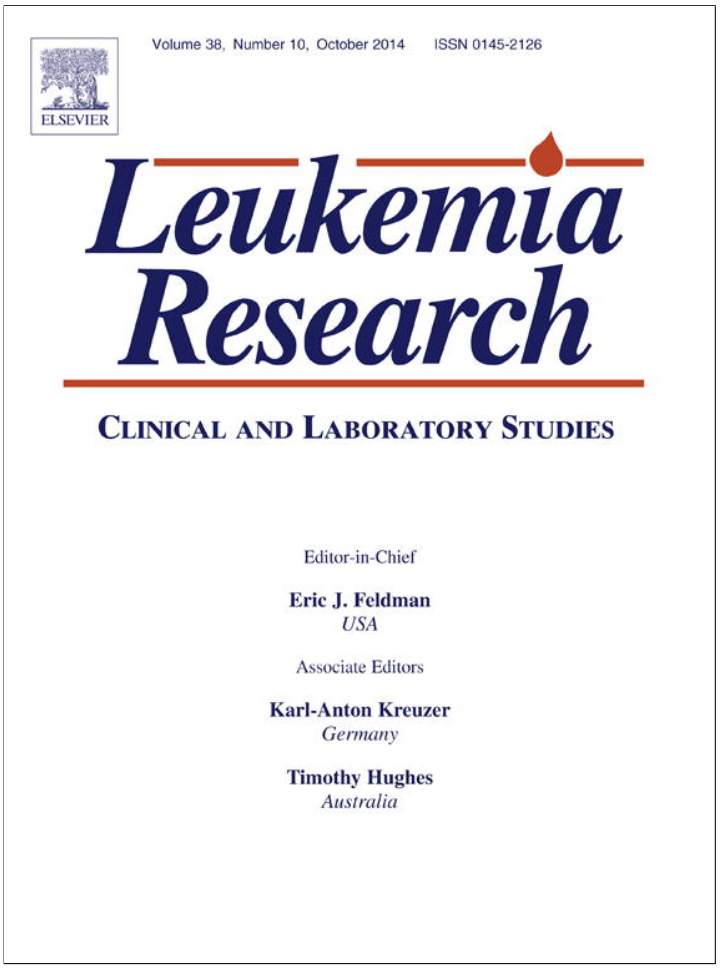

Leukemia Research 38 (2014) 1260–1265

Contents lists available at ScienceDirect

Leukemia Research

journal homepage: www.elsevier.com/locate/leukres

Erythrocyte membrane fatty acids in multiple myeloma patients

Artur Jurczyszyn[a,*,1], Jacek Czepiel[b,1], Joanna Gdula-Argasińska[c,1], Anna Czapkiewicz[d], Grażyna Biesiada[b], Mirosław Dróżdż[e], William Perucki[f], Jorge J. Castillo[g]

[a] Department of Hematology, University Hospital, Krakow, Poland
[b] Department of Infectious Diseases, Jagiellonian University Medical College, Krakow, Poland
[c] Department of Radioligands, Faculty of Pharmacy, Jagiellonian University Medical College, Krakow, Poland
[d] Faculty of Management, AGH University of Science and Technology, Krakow, Poland
[e] St. Anna Hospital, Miechów, Poland
[f] Students' Scientific Society, Jagiellonian University Medical College, Krakow, Poland
[g] Division of Hematologic Malignancies, Dana-Farber Cancer Institute, Boston, MA, USA

ARTICLE INFO

Article history:
Received 25 May 2014
Received in revised form 9 July 2014
Accepted 16 August 2014
Available online 25 August 2014

Keywords:
Multiple myeloma
Fatty acids
Erythrocyte membrane
Gas chromatography
Cluster analysis

ABSTRACT

Mounting data show that fatty acids (FA) and fatty acid synthase (FAS) function could be potential targets for multiple myeloma (MM) therapy. Our study aimed at comparing the FA composition of erythrocyte membranes of MM patients and healthy controls. MM patients had higher saturated FA and n-6 polyunsaturated FA (PUFA) and lower monounsaturated, n-3 PUFA and trans-FA indices than controls. The n-3/n-6 PUFA ratio was lower in MM patients and there was distinct clustering of variants of individual FA in MM patients. The FA content of erythrocyte membrane could serve as a diagnostic and/or predictive biomarker in MM.

1. Introduction

Multiple myeloma (MM) is the second most common hematological malignancy and accounts for approximately 1% of all malignant neoplasms. The incidence of MM is approximately 120,000 cases annually, with a global prevalence of over one million cases [1,2]. MM is characterized by a pathological, uncontrolled proliferation of malignant clonal plasma cells in the bone marrow, as well as the presence of monoclonal proteins in the plasma and/or urine.

Fatty acids (FA) play an important biological role not only for their energetic and storage functions but also for many physiological and pathological immunologic pathways. The potential influence of lipids on neoplastic development may be due to influence on the metabolism of neoplastic cells, the function of lipids as intercellular messengers, or as mediators of the inflammatory reaction. For example, increased fatty acid synthase (FAS) expression is observed in the cells of cancers including those of the large intestine, prostate, ovaries, endometrium, and breast [3]. FAS is important in the neoplastic process due to its influence on the proliferation of cells and the incorporation of lipids in the membranes of neoplastic cells, and can be a potential target for anticancer therapy, as previously described [4–6]. Wang et al. [7] observed increased expression of FAS in human myeloma lines as well as primary MM cells. Tirado-Velez et al. [8] demonstrated that inhibition of FA metabolism is associated with antiproliferative and apoptotic effects in human MM.

Recent studies have confirmed the importance of soluble cytokines and their receptors in the pathophysiology of MM. It has been shown that the level of soluble receptors for IL-6 (sIL-6R) is elevated in myeloma patients compared to healthy subjects, and its level correlates with disease severity [9]. Silvestris et al. [10] demonstrated that the abnormal up-regulation of apoptogenic

Abbreviations: FA, fatty acids; RBC, red blood cells; MM, multiple myeloma; SFA (SAT), saturated fatty acids; UNSAT, unsaturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic fatty acid; DHA, docosahexaenoic fatty acid; AA, arachidonic acid; LA, linoleic acid; ALA, α-linolenic acid; PBS, phosphate buffer saline; BHT, butylated hydroxytoluene; BF_3, boron trifluoride; GC, gas chromatography; FID, flame ionization detector; COX, cyclooxygenase (prostaglandin endoperoxide synthase).

* Corresponding author at: Department of Hematology, University Hospital, ul Kopernika 17, 31-501 Kraków, Poland. Tel.: +48 124247600.
E-mail address: mmjurczy@cyf-kr.edu.pl (A. Jurczyszyn).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.leukres.2014.08.009
0145-2126/

receptors, including both the Fas ligand (L) and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), by MM cells plays a role in the ineffective erythropoiesis and chronic exhaustion of the erythroid matrix seen in these patients [10,11].

Potentially valuable information can be gathered from the analysis of membrane lipid composition in RBC that provides a simple, suitable model for studying FA metabolism [12–16]. Therefore, accurate measurement of plasma and red blood cell FA could have important physiological and clinical implications. A better understanding of the distribution of FA in the erythrocyte membrane of patients will provide important insights into lipid metabolism and could guide future biomarker selection. Erythrocyte FA may be superior to plasma FA for reflecting long-term FA intake as they are less sensitive to recent intake and have a slower turnover rate [15,17–19]. Therefore, we hypothesized that the FA composition of erythrocyte membranes in patients with MM would differ from healthy controls.

2. Materials and methods

2.1. Reagents

Butylated hydroxytoluene (BHT), 14% BF_3 and FAME standards were purchased from Sigma–Aldrich (St. Louis, USA). Analytical grade chloroform, methanol and *n*-hexane were obtained from Merck (Darmstadt, Germany). Water (18.2 MΩ, TOC < 5 ppm) was ultrapurified and filtered through a Milli-Q Plus system filter (Millipore, Bedford, USA). Gases for chromatography of a 5.5 purity level were purchased from AirLiquide (Poland).

2.2. Patients

These patients were cared for at the Hematology clinic of the University Hospital in Krakow. Diagnosis of MM was based on current WHO criteria, patients with MM had greater than 10% plasma cells in BM aspirate and the presence of M-protein in the serum or urine [20]. Patients with MM were treated according to the guidelines of the Polish Myeloma Group. For patients under 65 years of age, first line treatment consisted of the CTD protocol (cyclophosphamide, thalidomide, and dexamethasone) followed by cyclophosphamide and G-CSF mobilization followed by autologous stem cell transplantation. In cases of disease recurrence, treatment consisted of bortezomib or lenalidomide based regimen plus steroids. Patients over 65 years of age were treated either with the MPT (melphalan, prednisone, and thalidomide) or VMP (bortezomib, melphalan, prednisone) protocols. In cases of disease progression lenalidomide and dexamethasone were given [21]. Exclusion criteria included the presence of acute or chronic inflammatory diseases.

2.3. Sample collection

Venous blood samples were collected in K_2-EDTA-containing tubes. Erythrocytes were separated from plasma by centrifugation (1500 × *g*, for 10 min) and washed with PBS. 10 μl of 0.05% BHT was added to each sample. The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee.

2.4. Isolation of cell membranes from erythrocytes

Hemoglobin-free erythrocyte membranes were prepared by hypotonic hemolysis at 4 °C in 10 mM Tris with pH 7.4. Membranes were then isolated by centrifugation (10,000 × *g* for 15 min) and washed several times to eliminate hemoglobin residues according to the method proposed by Graham [22].

2.5. Lipid analysis

Total lipid extraction from erythrocyte membranes was carried out with a solution of chloroform/methanol (2:1) [23]. The synthesis of fatty acid methyl esters of total lipids in the erythrocyte membranes was carried out with 14% BF_3 in methanol [24]. The FA methyl esters were analyzed using gas chromatography (Agilent 6890N) [25]. Chromatograph parameters were as follows: FID 260 °C, oven-start at 140 °C and ramp up to 240 °C. Column DB – 23 (60 m, 0.25 μm). Inlet temperature 250 °C, split 40:1, injection 1 μl. Fatty acid methyl esters were identified according to standards (Sigma, Supelco). The data were analyzed using ChemStation and Excel. Results were expressed in relative percentage of total FA.

2.6. Statistics

All data are presented as means ± SD. Differences between study groups were determined using the *F*-test, or the Mann–Whitney *U*-test if normality was not observed. Analysis of similarities between content of the erythrocyte membranes of healthy controls and MM patients was performed using clustering methods. Ward's method with Euclidean distance matrix was used to group a set of objects in such a way that objects in the same group are more similar to each other than to those in other groups. Calculations were performed using Statistica 10 (StatSoft®, Poland) software, and statistical significance was defined as $p \leq 0.05$.

3. Results

The study group included 43 patients with a pathological diagnosis of MM including 21 men and 22 women, with age ranging from 47 to 85 years (average 61 years), and with bone marrow plasmocytosis from 16% to 64% (average 36%). The average time from diagnosis was 2.4 years. In 55.9% of patients, we noted MM IgG kappa, in 20.9% there was light chain disease, in 11.6% IgA lambda, in 9.3% IgG lambda, and in 2.3% IgA kappa. According to ISS criteria, 41.9% of patients were classified as stage I, 16.2% as stage II and 41.9% as stage III. As of the present time, four patients (10%) have died. We also recruited 21 healthy controls, 13 men and 8 women, aged 35–78 years (average 52 years).

Comparison of erythrocyte membrane FA composition in patients with MM and controls are shown in Table 1. We identified 19 FA in erythrocyte membranes from MM patients and healthy subjects. We calculated total saturated FA (SFA), monounsaturated FA (MUFA), n-6 and n-3 polyunsaturated FA (n-6 PUFA, n-3PUFA) as well as *trans*-FA. Additionally, we calculated the n-3/n-6 ratio.

From our analysis we observed that FA profiles differ in the erythrocyte membranes of patients with MM when compared to the control group. The total SFA index was higher in MM patients than in controls (44% vs. 36%) with higher index of palmitic acid but lower index of stearic acid. The total MUFA index was lower in MM patients than in controls (16% vs. 26%) with higher indices of pentadecanoic and palmitoleic but lower index of oleic acid. The total n-6 PUFA index was higher in MM patients (33% vs. 26%) with higher indices of eicosadienoic, dihomo-γ-linolenic and arachidonic acid. The total n-3 PUFA index was lower in MM patients (7% vs. 9%) with higher indices of linolenic and eicosatrienoic acids but lower indices of EPA and docosapentanenoic acid. The total TFA index was lower in MM patients (0.7% vs. 1.5%). The n-3/n-6 ratio in erythrocyte membranes of MM patients was half than the control group (0.2 vs. 0.4).

To determine the covariant behavior of the measured variables, we analyzed such variables to identify which one best discriminates the FA profile in RBC membranes and plasma from healthy people and patients with MM (Fig. 1a and b). Variables were separated into four unique clusters. In healthy patients, RBC membrane cluster 1 consisted of n-3 FA (18:3n-3, 20:3n-3) and C22:4n6, while cluster 2 consisted of n-6 FA (18:3n-6, 20:2n-6, 20:3n-6). We observed grouping of certain medium chain FA variants (14:0, 14:1, 15:1 and 16:1) in cluster 3. In cluster 4 we observed grouping of FA variants 18:2n-6 and 20:4n-6. In contrast, variants 16:0, 18:0, and 18:1 grouped further away (Fig. 1a). In patients with MM, in contrast to the control group, grouping of n-3 FA in the first cluster consisted of the following variants: 18:3n-3, 20:3n-3 and 22:5n-3. n-6 FA variants dominated in the second cluster (18:3n-6, 20:2n-6, 20:3n-6). In the second cluster we also noted grouping of n-3 FA (DHA and EPA). Cluster 3 included medium-chain and long-chain FA variants. In cluster 4, we noted grouping of 18:2 n-6, 20:4 n-6, 16:0, 18:1, 18:0 and 16:1 FA (Fig. 1b).

4. Discussion

Our study is the first of its kind in that it evaluated the full FA profile of erythrocyte membranes in patients with MM. Motivation for this study came from suggestions that fish-based diet may have an effect on the course of hematological malignancies, including MM [12,26,27]. Further motivation came from recent studies

Table 1
Erythrocyte membrane fatty acid profile in 43 patients with myeloma and 21 healthy controls reported as means ± SD (% of peak area).

Fatty acids	Controls	Myeloma	*p* value
C14:0 myristic	2.0 ± 1.2 (0.2–5.3)	1.9 ± 1.3 (0.3–7.0)	NS
C16:0 palmitic	24.2 ± 3.5 (12.2–32.3)	36.4 ± 6.4 (21.0–53.2)	0.001
C18:0 stearic	10.8 ± 3.9 (4.5–18.0)	6.0 ± 3.6 (1.2–21.6)	0.001
Total SFA	36.6 ± 9.8 (26.7–48.8)	44.3 ± 5.9 (30.2–56.3)	0.001
C14:1n-5 myristoleic	1.9 ± 1.6 (0.1–6.9)	1.3 ± 1.1 (0.1–4.4)	NS
C15:1 pentadecanoic	0.1 ± 0.3 (0.0–1.4)	1.3 ± 1.5 (0.1–9.2)	0.001
C16:1 palmitoleic	3.5 ± 1.7 (1.0–7.4)	5.9 ± 3.4 (0.9–15.0)	0.002
C18:1n-9c oleic	19.6 ± 3.5 (12.4–25.7)	6.8 ± 2.2 (3.6–11.6)	0.001
C20:1n-9 eicosaenoic	0.8 ± 0.9 (0.0–3.2)	0.4 ± 1.0 (0.0–5.0)	NS
Total MUFA	25.9 ± 8.1 (19.8–33.8)	15.7 ± 4.6 (9.2–33.1)	0.001
C18:2n-6 linoleic	6.4 ± 2.5 (2.1–10.0)	5.9 ± 2.4 (0.0–12.8)	NS
C18:3n-6 γ-linolenic	2.2 ± 1.0 (0.0–4.0)	2.0 ± 1.1 (0.1–5.6)	NS
C20:2n-6 eicosadienoic	1.4 ± 2.2 (0.0–6.4)	2.7 ± 2.5 (0.0–10.2)	0.004
C20:3n-6 dihomo-γ-linolenic	1.7 ± 1.3 (0.0–3.4)	4.4 ± 2.8 (0.0–1.6)	0.001
C20:4n-6 arachidonic	14.2 ± 2.9 (9.2–22.0)	17.7 ± 3.5 (10.8–25.8)	0.001
C22:4n-6 adrenic	0.3 ± 0.1 (0.0–3.4)	0.1 ± 0.1 (0.0–0.1)	NS
Total PUFA n-6	26.3 ± 10.5 (18.4–35.0)	32.8 ± 4.3 (24.5–41.3)	0.001
C18:3n-3 linolenic	0.4 ± 0.5 (0.0–2.3)	0.9 ± 0.6 (0.1–2.8)	0.009
C20:3n-3 eicosatrienoic	0.1 ± 0.1 (0.0–1.1)	1.3 ± 0.6 (0.0–2.4)	0.001
C20:5n-3 EPA	5.4 ± 1.1 (2.3–9.4)	2.9 ± 1.2 (0.6–6.3)	0.001
C22:5n-3 docosapentaenoic	2.0 ± 0.7 (0.1–9.7)	0.3 ± 0.8 (0.0–4.3)	0.001
C22:6n-3 DHA	1.6 ± 0.2 (0.0–3.9)	1.3 ± 0.3 (0.0–2.6)	NS
Total PUFA n-3	9.4 ± 2.2 (6.8–15.0)	6.6 ± 3.5 (2.4–11.7)	0.001
C18:1n-9t elaidic	1.5 ± 1.1 (0.0–4.1)	0.7 ± 0.8 (0.0–2.6)	0.004
Total *trans* fatty acids	1.5 ± 1.1 (0.0–4.1)	0.7 ± 0.8 (0.0–2.6)	0.004
n-3/n-6 ratio	0.4 ± 0.2 (0.2–0.7)	0.2 ± 0.1 (0.1–0.3)	0.001

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. % peak area calculated from fatty acid methyl esters.

investigating the role of pro-inflammatory cytokines in the progression and prognosis of such patients [28,29]. Our major findings are as follows: (1) The profile of FA in erythrocyte membranes differs significantly in MM patients compared to controls, (2) the n-3/n-6 ratio of the RBC membrane was significantly lower and the SFA index was significantly higher in MM patients than in controls, and (3) there is distinct clustering of the variants of individual FA in the erythrocyte membranes of MM patients compared to controls.

Among the potential risk factors that have been investigated, FA are thought to alter inflammatory responses and contribute to lymphomagenesis. FA are essential components of cell membranes and affect eicosanoid and cytokine production. In particular, the ratio of n-3 and n-6 polyunsaturated fatty acids (PUFAs) may play a key role in influencing non-Hodgkin lymphoma risk, as these essential FA compete in the release of anti- and pro-inflammatory eicosanoids, respectively, and n-3 PUFAs suppress pro-inflammatory cytokine production [30]. Previous studies have shown a positive association of dietary SFA intake with higher NHL risk, possibly through pro-inflammatory mediator production [31]. For other cancers and diseases, n-3 PUFAs, found largely in fish oils, have been shown to be protective, while n-6 PUFAs, abundant in a typical Western diet, appear to be pro-inflammatory [32].

In our study, the n-3/n-6 ratio in erythrocyte membranes was lower in patients with MM when compared to the control group. n-3 PUFAs exert anti-inflammatory actions by inhibiting pro-inflammatory signal transduction pathways whereas n-6 PUFAs facilitate inflammation by serving as a precursor of pro-inflammatory eicosanoids [33,34]. Moreover, we observed significantly lower EPA/n-6 PUFA and n-3/n-6 ratios in the RBC membranes of MM patients than in the control group, which might be associated with n-6 PUFA-related inflammatory complications. We observed low levels of n-3 PUFA in the erythrocyte cell membranes in patients with MM compared to healthy subjects (Table 1). This observation could be attributed to the lack of a recommendation to increase dietary n-3 FA. The cytokine network is important in the development and survival of tumor cells as well as progression of MM. Cancer cells arise in every human being throughout life; a well-functioning immune system prevents their survival. The human diet is dependent on each individual. Perhaps an imbalance of pro- and anti-inflammatory cytokines, caused by changes in the relative n-3/n-6 ratio, is one of the factors that may initiate the survival and further growth of MM cells in the human body.

SFAs, and notably long-chain SFAs, have been associated with inflammation. Although the mechanism is unclear, toll-like receptor (TLR) 4, TLR2, the synthesis of ceramides, the formation of lipid rafts and fetuin seem to be implicated. On the other hand, medium-chain SFAs have shown beneficial health effects including suppression of body fat accumulation, leading to decreased obesity [35]. In our study, index SFA was significantly higher in the RBC membranes of patients with MM than in healthy people. Stearic acid is converted into oleic acid by the liver microsomal desaturase system. Increased D9 desaturation and low saturation index have been observed in colorectal and bronchogenic carcinoma, lymphoma, leukemia and malignant liver neoplasms. Also, a decrease in the saturation index of both erythrocytes and leukocytes in patients with leukemia has been identified [16,28,36]. Pandey et al. described a concept that cancer is a systemic disease, and that the decrease in saturation index is not type-specific but is associated with neoplasia in general [37].

In our study, we observed distinct clustering of the variants of individual FA in the erythrocyte membranes of MM patients compared to controls. In healthy patients, RBC membrane clusters consisting of n-3 and n-6 PUFAs were closer than in MM. This might indicate a normal function of enzymes that are involved in the elongation and desaturation of PUFAs [37]. In patients with MM, grouping of n-3 FA was divided into two clusters (Fig. 1a and b). A clear interpretation of these results is difficult, but probably indicates a malfunction of enzymes such as desaturases and elongases, and subsequent changes in the FA desaturase (FADS) gene expression, or polymorphisms in patients with MM. Associations between FADS1 and FADS2 haplotypes and distinct PUFA

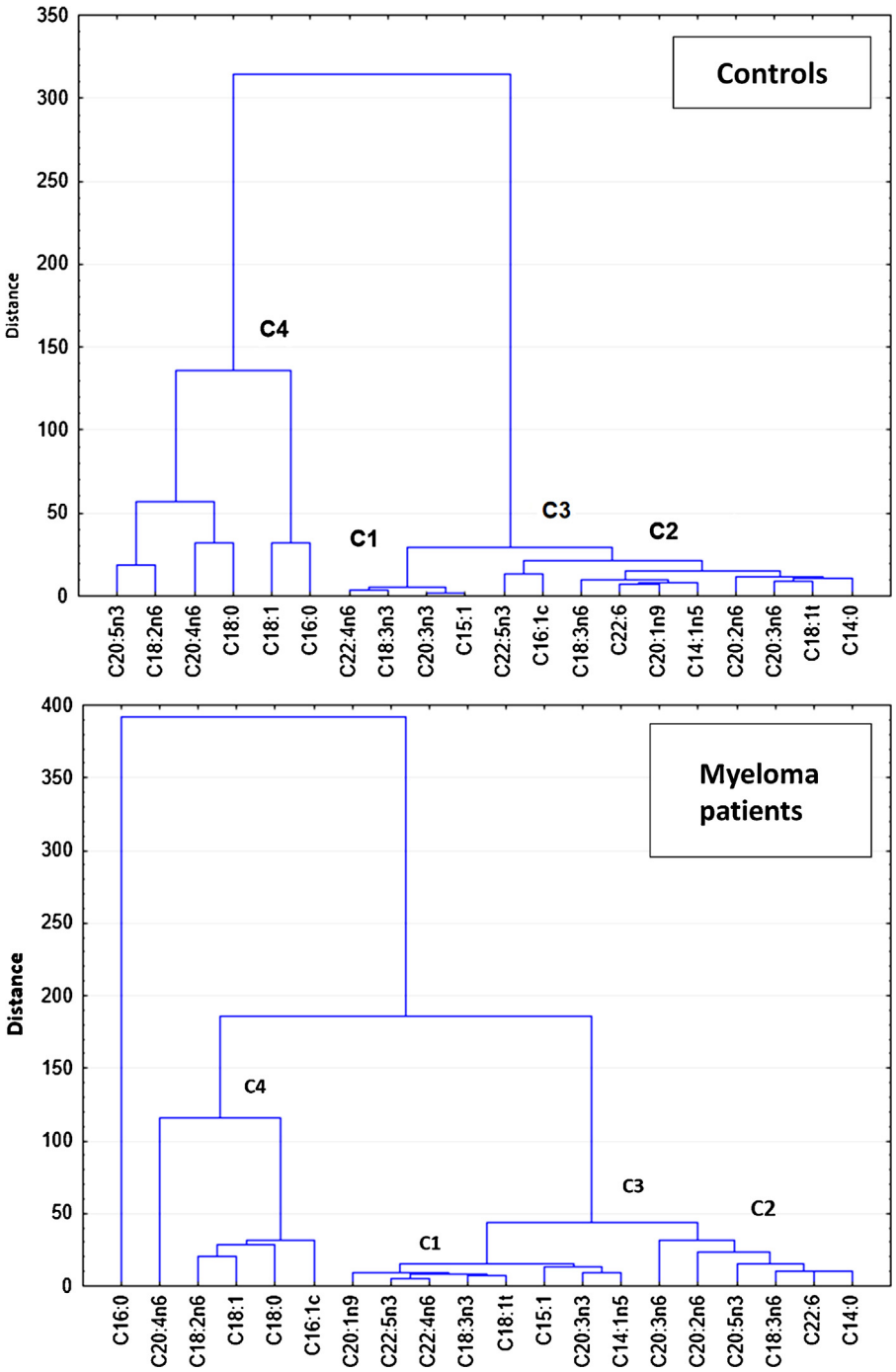

Fig. 1. Cluster analysis of the FA profile in RBC membranes from healthy people (control) and from myeloma multiple patients (MM). Data were segregated into four unique clusters of variables by hierarchical cluster analysis.

composition of erythrocyte membranes, particularly arachidonic and dihomo-γ-linolenic acid, have been observed [37]. These results are hypothesis generating, and deserve further attention.

The FA composition of the cell membrane is influenced by both dietary intake and metabolic pathways. Lipid peroxidation under conditions of oxidative stress can also contribute to alterations in membrane structure and function [19,38,39]. In our study, the levels of erythrocyte EPA and docosapentaenoic acid in MM patients were substantially lower than the levels of healthy subjects (Table 1). These data suggest that there may be reduced endogenous synthesis of EPA from ALA in MM patients probably due to decreased functionality of desaturase and elongase enzymes. Additionally, the levels of elaidic acid, a *trans*-FA, were higher in controls than in MM patients (Table 1). *Trans*-FA intake may influence factors related to cancer risk such as systemic inflammation, insulin resistance, and adiposity, but the association between *trans*-FA intake and cancer risk has not been extensively studied [40].

Differences in the FA profile of patients with MM compared with healthy people, especially the n-3/n-6 ratio may be related to reduced supply of n-3 FA in the diet, chronic inflammation, or the related net of lipid derivatives from n-6 FA in MM patients. Moreover, in patients with MM, abnormalities may be present in the bone marrow FA fat, which can cause inflammation and can

result in disturbances in bone marrow stromal cells. The bone marrow is a complex microenvironment, in which a variety of cell types share a common space. MM cells release cytokines and growth factors that affect the cells in their vicinity. Bone marrow fat (BMF) is now considered to play an important role within the bone marrow microenvironment. BMF is not comparable to other fat depots such as subcutaneous or visceral tissues. Recent studies on bone marrow adipocytes have shown that besides acting as storage cells, these cells also secrete adipokines, like leptin and adiponectin. Moreover, bone marrow adipocytes share the same precursor with osteoblasts, the mesenchymal stem cell. It is now well established that high BMF is associated with weak bone mass in osteoporosis, especially during aging and anorexia nervosa [41,42]. n-6 FA affect inflammatory processes mainly through activation and changes in eicosanoid synthesis and signal transduction. Two hypotheses may define these molecular mechanisms. The first, suggests that n-6 FA regulate the transcription of cell inflammatory genes by up-regulating the activity of the nuclear factor NFκB, predicting changes in the levels of adhesion receptors and chemokine expression. The second hypothesis proposes that AA may compete with the n-3 FA for cyclooxygenase enzymes (COX-1 and COX-2 [12,33]).

Hematopoiesis is a tightly regulated process that allows the body to both maintain physiological levels of cells and respond to pathological conditions. Stem cell migration is a common feature of hematopoiesis. These migratory processes, especially the egress of stem cells from bone marrow, are regulated by various agents, including cytokines and chemotherapeutics, and more recently also endocannabinoids derived mainly from AA. *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG) stimulate hematopoiesis and stem cell migration, respectively, by synergizing with colony-stimulating factor (CSF), interleukin-3 (IL-3) and erythropoietin through CB2 receptor. 2-AG has also been shown to increase CFU-GEMM (colony-forming unit: granulocyte, erythrocyte, macrophage, megakaryocyte)-induced colony formation and cell migration in a receptor CB1- and CB2-dependent manner, whereas AEA increases colony yield but inhibits cell migration via CB1 and CB2 [43].

In patients with MM, the n-3/n-6 ratio was significantly decreased in comparison to the healthy group. Such observation suggests that the former group does not consume sufficient n-3 unsaturated fatty acids in their diet. n-3 PUFAs could be used as so-called "nutraceuticals" to modify the composition and organization of membranes to selectively initiate a change in cellular function. n-3 PUFAs have been shown to attenuate growth and induce apoptosis in a variety of human cancer cell lines. Recent findings indicate that n-3 PUFAs act synergistically with chemotherapeutic agents and may also be used to enhance tumor radiosensitivity [44]. Furthermore, these acids act as ligands for nuclear peroxisome proliferator-activated receptors (PPAR) that attenuate transcription of NF-κB dependent genes. Because of the potential benefits of n-3 FA and their derivatives, additional studies should study the addition of these products to the diets of cancer patients. Recently, several studies have demonstrated that PPAR is also expressed in human MM cells and PPARγ ligands play a role in the apoptosis of MM cells. The ligands of PPARγ may represent novel agents for treatment of human MM [45,46].

Modifications of erythrocyte membrane FA composition are an early indicator of lipid disorders, and manifest themselves earlier than changes in plasma lipoproteins. Our study underscores the important role of FA may have in the process of carcinogenesis. Deficiency of endogenous n-3 PUFAs may lead to changes in the physicochemical properties of cell membranes, activation of the synthesis of pro-inflammatory and vasoconstrictive eicosanoids, and finally induction of a pro-inflammatory condition, which may be important in the development and progression of neoplastic diseases [45]. The ability to control, and possibly modify, an individual's FA profile could prove to be useful as MM prophylaxis, for example. One important element of such prevention could be a diet rich in n-3 fatty acids.

Further research is needed, especially comparing lipid changes with relation to the duration and progression of myeloma. The FA content of erythrocyte membranes in MM patients could give us a better understanding of the lipid biochemistry and molecular mechanism of MM. This information has potential implications as a diagnostic tool, as well as for elucidating the biochemical pathways involved in response to chemotherapy. The future pipeline for MM agents is very promising. Perhaps the development of new treatments in combination with an appropriate diet rich in n-3 FA, acting as lipid mediators or ligands for transcription factors PPARs and NFκB could translate into response and survival benefits in patients with MM.

Authors contribution

Conceived and designed the experiments: AJ, JC, JGA. Performed the experiments: JGA. Analyzed the data: JGA, JC, AC, JJC. Contributed reagents/materials/analysis tools: AJ, JC, JGA, GB, MD. Statistics: AC, JGA. Wrote the paper: JGA, JC, JJC, WP.

Conflict of interest

The authors otherwise disclose no conflicts of interest.

Acknowledgment

This project was partially supported by National Science Centre, Poland to the author Joanna Gdula-Argasińska DEC-2011/01/B/NZ7/00038.

References

[1] Jurczyszyn A, Zaucha JM, Machaczka M, Grosicki S, Dybko J, Czepiel J, et al. Necessary diagnostic requirements and therapeutic recommendations in multiple myeloma. Przegl Lek 2013;70:744–53.
[2] Durie BG, Kyle RA, Belch A, et al. Myeloma management guidelines: a consensus report from the Scientific Advisors of the International Myeloma Foundation. Hematol J 2003;4:379–98.
[3] Rashid A, Pizer ES, Moga M, Milgraum LZ, Zahurak M, Pasternack GR, et al. Elevated expression of fatty acid synthase and fatty acid synthetic activity in colorectal neoplasia. Am J Pathol 1997;150:201–8.
[4] Menendez JA, Lupu R. Fatty acid synthase-catalyzed de novo fatty acid biosynthesis: from anabolic energy-storage pathway in normal tissues to jack-of all-trades in cancer cells. Arch Immunol Ther Exp 2004;52:414–26.
[5] Gabrielson EW, Pinn ML, Testa JR, Kuhajda FP. Increased fatty acid synthase is a therapeutic target in mesothelioma. Clin Cancer Res 2001;7:153–7.
[6] Pizer ES, Pflug BR, Bova GS, Han WF, Udan MS, Nelson JB. Increased fatty acid synthase as a therapeutic target in androgen independent prostate cancer progression. Prostate 2001;47:102–10.
[7] Wang W, Zhao X, Wang H, Liang Y. Increased fatty acid synthase as a potential therapeutic target in multiple myeloma. J Zhejiang Univ Sci B 2008;9:441–7.
[8] Tirado-Velez JM, Joumady I, Saez-Benito A, Cozar-Castellano I, Perdomo G. Inhibition of fatty acid metabolism reduces human myeloma cells proliferation. PLOS ONE 2013;7:1–13.
[9] Jurczyszyn A, Czepiel J, Biesiada G, Gdula-Argasińska J, Cibor D, Owczarek D, et al. HGF, sIL-6R and TGF-β1 play a significant role in the progression of multiple myeloma. J Cancer 2014;5:518–24.
[10] Silvestris F, Cafforio P, Tucci M, Dammacco F. Negative regulation of erythroblast maturation by Fas-L$^+$/TRAIL$^+$ highly malignant plasma cells: a major pathogenetic mechanism of anemia in multiple myeloma. Blood 2002;99:1305–13.
[11] Frassanito MA, Silvestris F, Silvestris N, Camarda G, Iodice G, Dammacco F. Fas/Fas ligand (Fas-L)-deregulated apoptosis and IL-6 insensibility in highly malignant myeloma cells. Clin Exp Immunol 1998;114:711–20.
[12] Fernandez E, Chatenoud L, La Vecchia C, Negri E, Franceschi S. Fish consumption and cancer risk. Am J Clin Nutr 1999;70:85–90.
[13] Martinelli N, Girelli D, Malerba G, Guarini P, Illig T, Trabetti E, et al. FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. Am J Clin Nutr 2008;88:941–9.

[14] Kelavkar UP, Hutzley J, McHugh K, Allen KGD, Parwani A. Prostate tumor growth can be modulated by dietary targeting the 15-lipoxygenase-1 and cyclooxygenase-2 enzymes. Neoplasia 2009;11:692–9.
[15] Zietemann V, Kröger J, Enzenbach C, Jansen E, Fritsche A, Weikert C, et al. Genetic variation of the FADS1 FADS2 gene cluster and n-6 PUFA composition in erythrocyte membranes in the European Prospective Investigation into Cancer and Nutrition-Potsdam study. Br J Nutr 2010;104:1748–59.
[16] Lands B. Consequences of essential fatty acids. Nutrients 2012;4(9):1338–57.
[17] Patel PS, Sharp SJ, Jansen E, Luben RN, Khaw K-T, Wareham NJ, et al. Fatty acids measured in plasma and erythrocyte-membrane phospholipids and derived by food-frequency questionnaire and the risk of new-onset type 2 diabetes: a pilot study in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Norfolk cohort. Am J Clin Nutr 2010;92:1214–22.
[18] Kröger J, Zietemann V, Enzenbach C, Weikert C, Jansen EHJM, Döring F, et al. Erythrocyte membrane phospholipid fatty acids, desaturase activity, and dietary fatty acids in relation to risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)–Potsdam Study. Am J Clin Nutr 2011;93:127–42.
[19] Perona JS, Gonzalez-Jimenez E, Aguilar-Cordero MJ, Sureda A, Barcelo F. Structural and compositional changes in erythrocyte membrane of obese compared to normal-weight adolescents. J Membr Biol 2013;246:939–47.
[20] Kyle RA, Rajkumar SV. Criteria for diagnosis, staging, risk stratification and response assessment of multiple myeloma. Leukemia 2009;23:3–9.
[21] Dmoszyńska A, Walter-Croneck A, Usnarska-Zubkiewicz L, et al. Guidelines of Polish Myeloma Group concerning diagnosis and therapy of multiple myeloma and other plasmacytic dyscrasias for 2013. Acta Haematol Pol 2013;44:3–47.
[22] Graham J. Purification of human erythrocyte 'ghosts'. In: Harris JR, Graham J, Rickwood D, editors. Cell Biology Protocols. John Wiley & Sons, Ltd.; 2006. p. 137–8.
[23] Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 1957;226:497–509.
[24] Metcalfe LD, Schmitz AA. The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal Chem 1961;33:363–7.
[25] Li Y, Watkins BA. Conjugated linoleic acids alter bone fatty acid composition and reduce ex vivo prostaglandin E2 biosynthesis in rats fed n-6 or n-3 fatty acids. Lipids 1998;33:417–21.
[26] Tavani A, Pregnolato A, Negri E, Franceschi S, Serraino D, Carbone A, et al. Diet and risk of lymphoid neoplasms and soft tissue sarcomas. Nutr Cancer 1997;27:256–60.
[27] Fritschi L, Ambrosini GL, Kliewer EV, Johnson KC, Canadian Cancer Registries Epidemiologic Research Group. Dietary fish intake and risk of leukaemia, multiple myeloma, and non-hodgkin lymphoma. Cancer Epidemiol Biomarkers Prev 2004;13:532–7.
[28] Mehtap O, Atesoglu EB, Tarkun P, Hacihanefioglu A, Dolasik I, Musul MM. IL-21 and other serum proinflammatory cytokine levels in patients with multiple myeloma at diagnosis. J Postgrad Med 2014;60:141–4.
[29] Anderson KC, Carrasco RD. Pathogenesis of myeloma. Annu Rev Pathol 2011;6:249–74.
[30] Ward MH, Zahm SH, Weisenburger MDD, Gridley MG, Cantor KP, Saal MRC, et al. Dietary factors and non-Hodgkin's lymphoma in Nebraska (United States). Cancer Causes Control 1994;5:422–32.
[31] Hosgood III HD, Baris D, Zahm SH, Zheng T, Cross AJ. Diet and risk of multiple myeloma in Connecticut women. Cancer Causes Control 2007;18:1065–76.
[32] Morimoto Y, Conroy SM, Ollberding NJ, Henning SM, Franke AA, Wilkens LR, et al. Erythrocyte membrane fatty acid composition, serum lipids, and non-Hodgkin's lymphoma risk in a nested case–control study: the multiethnic cohort. Cancer Causes Control 2012;23:1693–703.
[33] Greene ER, Huang S, Serhan CN, Panigrahy D. Regulation of inflammation in cancer by eicosanoids. Prostaglandins Other Lipid Mediat 2011;96:27–36.
[34] Davidson J, Rotondo D, Rizzo MT, Leaver HA. Therapeutic implications of disorders of cell death signaling: membranes, micro-environment, and eicosanoid and docosanoid metabolism. Br J Pharmacol 2012;166:1193–210.
[35] Ruiz-Núñez RS, Kuipers MF, Luxwolda DJ, De Graaf BB, Dijck-Brouwer DAJ, Muskiet FAJ. Saturated fatty acid (SFA) status and SFA intake exhibit different relations with serum total cholesterol and lipoprotein cholesterol: a mechanistic explanation centered around lifestyle-induced low-grade inflammation. J Nutr Biochem 2014;25:304–12.
[36] Pandey M, Khatri AK, Dubey SS, Gautam A, Shukla VK. Erythrocyte membrane stearic to oleic acid ratio in carcinoma of the gallbladder: a preliminary study. Eur J Surg Oncol 1998;24:43–6.
[37] Rzehak P, Heinrich J, Klopp N, Schaeffer L, Hoff S, Wolfram G, et al. Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. Br J Nutr 2009;101:20–6.
[38] Takkunen M, Agren J, Kuusisto J, Laakso M, Uusitupa M, Schwab U. Dietary fat in relation to erythrocyte fatty acid composition in men. Lipids 2013;48:1093–102.
[39] Yawata Y. Cell Membrane: The Red Blood cell as a Model, vol. 46. Weinheim: Wiley-VCH Verlag GmbH & Co.; 2003. p. 27–46, 379–414.
[40] Laake I, Carlsen MH, Pedersen JI, Weiderpass E, Selmer R, Kirkhus B, et al. Intake of trans fatty acids from partially hydrogenated vegetable and fish oils and ruminant fat in relation to cancer risk. Int J Cancer 2013;132:1389–403.
[41] Hardouin P, Pansini V, Cortet B. Bone marrow fat. Joint Bone Spine 2014, http://dx.doi.org/10.1016/j.jbspin.2014.02.013 [Epub ahead of print].
[42] Bermeo S, Gunaratnam K, Duque G. Fat and bone interactions. Curr Osteoporos Rep 2014;12(2):235–42.
[43] Galve-Roperha I, Chiurchiùb V, Díaz-Alonsoa J, Bari M, Guzmána M, Maccarrone M. Cannabinoid receptor signaling in progenitor/stem cell proliferation and differentiation. Prog Lipid Res 2013;52:633–50.
[44] Wendel M, Heller AR. Anticancer actions of omega-3 fatty acids – current state and future perspectives. Anticancer Agents Med Chem 2009;9:457–70.
[45] Garcia-Bates TM, Bernstein SH, Phipps RP. Peroxisome proliferator-activated receptor; overexpression suppresses growth and induces apoptosis in human multiple myeloma cells. Clin Cancer Res 2008;14:6414–25.
[46] Dessi M, Noce A, Bertucci P, Manca di Villahermosa S, Zenobi R, Castagnola V, et al. Atherosclerosis, dyslipidemia, and inflammation: the significant role of polyunsaturated fatty acids. ISRN Inflamm 2013;2013:1–13.