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RESEARCH ARTICLE

MOLECULAR SUBTYPES AND DIETARY PATTERNS IN BREAST CANCER PATIENTS: A LATENT CLASS ANALYSIS

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ABSTRACT: Breast cancer (BC) is the second most common cancer worldwide, with over 2,300,000 new cases estimated per year. Diet has been identified as a modifiable risk factor for BC development and prognosis. The Mediterranean diet (MD) has shown to be inversely associated with chronic diseases including BC. The aim of the present study was to assess dietary patterns according to BC molecular subtypes in a subgroup of patients at their baseline visit of a lifestyle trial conducted by our institute. A principal component analysis (PCA) was conducted to assess the best dimensional space where to summarize dietary information. An explorative unsupervised automatic clustering technique was performed to identify diet-risks groups. Final groups were analyzed as dietary patterns and comparisons made by synthetic statistics with univariable analysis. The first PCA factor was characterized mainly by vegetables (27.6%), nuts & extra-virgin olive oil (EVOO) (16.7%), and sweet & sugars (11.8%). Legumes and fats separately represented just over 10% of the first PCA factor. The second factorial axis was represented mainly by cereals (40.9%), sweet & sugars (20.2%) and nuts & EVOO (15.4%). PCA showed different behaviors between dietary variables in each molecular subtype, especially among patients with triple negative TNBC (n = 37) the strongest contribution to the first PCA factor was given by sweet & sugars (20.7%), then vegetables (17.1%), fruits (11.9%) and legumes (11.0%) while animal proteins (24.3%), nuts & EVOO (16.0%), fruits (14.1%) and fish (12.1%) determined the second factor. From k- means, three clusters of patients were found. Cluster 1 (Healthy pattern) was associated with healthier dietary habits compared with the other two groups, with approximately twice the vegetables (204 grams vs. 119 grams for cluster 2 and 95 grams for cluster 3, $p < 0.01$). Cluster 2 (Western pattern) was characterized by greater refined cereals and animal protein, sedentary behavior and higher body mass index (BMI) and central obesity (35% with ≥30 kg/m^2 compared with 13% in cluster 1 and 25% in cluster 3. Cluster 3 (Ultraprocessed pattern) was characterized by greater intakes of sweet & sugars and non-EVOO fat, cluster 2 was composed mainly of Luminal BC subtype, while TNBC were found mostly in cluster 1 and cluster 3.
Our findings revealed three main dietary risk group by BC patients at the baseline visit of a lifestyle Trial: a healthy dietary group (cluster 1), a western diet group (cluster 2) and an ultra-processed food diet group (cluster 3). The former is considered part of a healthy Mediterranean diet which is known to improve the metabolic and hormonal risk factors for BC and reduce total mortality. A concern emerged for the high-risk group of TNBC patients who tend to be younger and appeared to consume more sweets and fats which are known risk factors for chronic diseases and poor cancer prognosis.

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Impact statement: Different dietary patterns emerged according to Breast Cancer molecular subtypes through a principal component analysis (PCA) that analyzed dietary information in a lifestyle trial conducted by our institute; moreover, an explorative unsupervised automatic clustering technique was performed to identify diet-risks groups.

Key words: *breast cancer; dietary patterns; molecular subtypes; Latent class analysis; Cluster analysis.*

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INTRODUCTION

Breast cancer (BC) is the second most common cancer worldwide, with over 2,300,000 new cases estimated in 2020 (1) and the fifth cause of cancer death globally (683,100 deaths estimated in 2020). In women, it is the most common neoplasm overall and the leading cause of cancer death. Since the 1990s, a gradual and constant reduction in mortality has been observed due to the implementation of screening programs and the availability of effective diagnostic-therapeutic approaches.

In Italy, approximately 55,700 new cases of BC are estimated each year (2). The risk of developing BC increases exponentially with age, particularly in the post-menopausal period. Recent data showed an increase in BC incidence in Italy (+0.5% compared to 2020) due to a higher life expectancy and early detection due to screening (2). The incidence is higher in the Northern than in Central Italy and the Islands due to different implementation and diffusion of screening programs and distribution of risk factors. In Italy as well BC represents the leading cause of cancer death in women with 12,500 deaths in 2021 although there has been a constant reduction in mortality over the last decade (estimated -2.2% per year) (2).

BC can be classified into the following four subtypes according to the expression of estrogen receptors (ER), progesterone receptors (PgR), cellular proliferation index (Ki67) and overexpression/amplification of the human epidermal growth factor receptor 2 (HER2):

1. Luminal A-like: low grade, with high expression level of ER/PgR, HER2 negative and Ki67;
2. Luminal B-like: high grade, variable ER/PgR expression, high Ki67, further divided into HER2 positive or negative;
3. HER2 positive tumors: ER/PgR negative and HER2 positive;
4. Triple negative breast cancer (TNBC): ER, PgR and HER2 negative (none expressed).

TNBC accounts for approximately 15% of BC cases is more frequent in young and obese women, often carries BRCA1 mutations and has the worst prognosis among all BC subtypes.

The absence of a targeted therapy, the tendency to metastasize to the central nervous system and visceral organs (3, 4) and the higher risk of relapse and distant recurrence represent the main factors explaining poor prognosis of TNBC (4).

Among modifiable risk factors for BC morbidity and mortality, diet plays a key role. It has been estimated that 30-50% of BC deaths could be avoided by dietary modifications alone (5). The dietary recommendations from international cancer institutions such as the World Cancer Research Fund (WCRF) suggest for the primary and secondary prevention of BC a diet rich in whole grains, vegetables, fruits, and legumes, while limiting the consumption of fast foods and processed foods, red and processed meat, sweets, sugar-sweetened drinks, and alcohol (6). Specifically for BC survivors the guidelines recommend a diet rich in soy foods and fiber as they have been significantly inversely associated with BC outcomes (recurrence, cancer-specific mortality, and all-cause mortality) (6, 7). The Mediterranean diet is one of the healthiest dietary patterns. The Mediterranean diet is considered by high consumption of plant-based foods (*i.e.*, vegetables, fruits, whole grains, legumes, nuts, olives and olive oli as the main source of fat); low intake of red and processed meat, saturated fats, and refined sugars; low to moderate consumption of dairy products; moderate consumption of fish; and moderate intake of alcohol (mostly red wine) with meals (8). Adherence to the Mediterranean diet has been inversely associated with multiple chronic disease, including cardiovascular disease and its risk factors, diabetes, and cancer, in epidemiological investigations and clinical trial (9-13). In the Nurses' Health Study, a healthy diet reach in

fruits and vegetables has been inversely associated with decreased BC risk particularly for the most aggressive BC subtypes (14).

The aim of the present study was to assess baseline dietary patterns by principal component analysis (PCA) overall and according to molecular subtypes in BC patients participating in a lifestyle trial in Italy (15).

MATERIALS AND METHODS

Patients

This study included 223 women (age range 30-70 years) with a BC diagnosis (stages I-III) participating in an ongoing multicenter randomized controlled trial of the effect of a treatment program of dietary modification, physical activity, and vitamin D supplementation (DEDiCa Study) on BC recurrence (21). The study protocol was approved by the Italian Ministry of Health, Italian Medicine Agency (AIFA) and the Ethic Boards of each recruiting hospital (ClinicalTrials.gov NCT02786875). Participants were recruited and followed up in national cancer institutes or oncologic departments of hospitals located in Southern and Northern Italy: Istituto Nazionale Tumori IRCCS Fondazione G. Pascale (Naples), Clinica Mediterranea (Naples), Villa Betania (Naples), Ospedale dei Colli Monaldi (Naples), Cannizzaro Hospital (Catania), San Vincenzo Hospital (Taormina), Istituto Nazionale Tumori IRCCS CRO (Aviano). Eligible participants were found through surgical lists of participating hospitals. Patients were contacted by phone and offered to learn more about the study during group information sessions. Informed consent was obtained at baseline from all participants randomized in the study. The main inclusion criteria were women with primary diagnosis of histologically confirmed BC within 12 months from diagnosis and no history of any cancer except non-melanoma skin cancer. The demographic characteristics of the 223 participants are shown in **Table 1**.

Dietary variables

Dietary data were derived from 7-day food records completed by participants 7 days before their baseline study visit, reviewed by trained dietitians and analyzed with the nutritional analysis software Winfood. Food and food groups were calculated in grams per 1000 Kilo calorie (Kcal) of

Table 1. *Distribution of main BC patients' characteristics.*

CHARACTERISTICS	N = 223
Age (years)	
Mean (SD)	52 (9)
Cancer stage	
IA-2A	166 (74%)
2B	25 (11%)
3A-3B	32 (14%)
Molecular subtypes	
Luminal	145 (65%)
Her2+	41 (18%)
Triple Negative	37 (17%)
Waist circumference (cm)	
Mean (SD)	94 (14)
Body mass index, BMI (kg/m²)	
Mean (SD)	27.2 (5.8)
<25	95 (43%)
25-29	70 (31%)
≥30	58 (26%)
Education (years)	
Mean (SD)	12.7 (4.6)
PREDIMED score (out of 14 points)	
Mean (SD)	8.21 (1.92)
Potatoes (grams/1000 Kcal/day)	
Mean (SD)	14 (12)
Fish (grams/1000 Kcal/day)	
Mean (SD)	31 (24)
Vegetables (grams/1000 Kcal/day)	
Mean (SD)	131 (72)
Fats and oil dressings (grams/1000 Kcal/day)	
Mean (SD)	1.35 (1.79)
Animal protein (grams/1000 Kcal/day)	
Mean (SD)	49 (26)
Cereals and pizza (grams/1000 Kcal/day)	
Mean (SD)	110 (32)
Legumes (grams/1000 Kcal/day)	
Mean (SD)	19 (25)
Fruits, fruit drinks and jams (grams/1000 Kcal/day)	
Mean (SD)	226 (105)
Sweets and sugars (grams/1000 Kcal/day)	
Mean (SD)	32 (31)
Nuts and EVOO (grams/1000 Kcal/day)	
Mean (SD)	14 (9)

Abbreviations: EVOO: extra virgin olive oil; HER2: human epidermal receptor-2; PREDIMED: Prevencion con Dieta Mediterranea (Spanish study); SD: standard deviation.

total energy intake and included animal proteins (meat, processed meat, eggs); cereals and pizza (whole and refined grains); fats and dressings (butter, lard, vegetable oils but not olive oil); fish and seafood; fresh fruit, fruit jams and fruit juices; legumes (including soya beans); nuts and extra virgin olive oil (EVOO); potatoes; sugar and sweets (including commercial sweet beverages); and vegetables (non-starchy vegetables).

Adherence to the Mediterranean diet (MD) was also summarized by the 14-item PREDIMED (Prevencion con Dieta Mediterranea) questionnaire administered by the study staff. This questionnaire was created by the PREDIMED study group in Spain to investigate adherence to the MD of participants in the dietary intervention trial (16). The PREDIMED questionnaire consists of 14 questions in total: 12 questions on food quantities and frequency of consumption (extra-virgin olive oil, vegetables, fruit, red or processed meats, butter, soda drinks, legumes, fish, commercial sweets, nuts, wine, sofrito sauce) and 2 general questions on intake habits regarding olive oil and meat. Each question included two possible answers and scores: 1 score for "yes" answer, indicating greater adherence to the MD and 0 for "no" answer. The PREDIMED final score ranges from 0 to 14 where 14 represented the highest adherence to MD.

Statistical analysis

Main BC patients' characteristics were analyzed as means and standard deviation (SD) for numerical variables and count (with percentages) for categorical variables. A univariable analysis was performed between molecular subtypes and the main variables investigated in the present analysis by Chi-Square test.

A multiple correlation matrix was performed between food groups (heat map, **Figure 1**) and a PCA was conducted to summarize dietary information about many food groups into a small set of principal components or dietary patterns. This analysis was performed in the overall sample and by molecular subtypes with the aim of assessing potential differences in dietary patterns among subgroup (**Figure 2**). An explorative unsupervised automatic clustering technique by k-means methodology was performed to identify diet-risks groups (**Figure 3**). A Cluster tendency statistic (Hopkins' statistic) was reported as index of goodness of cluster performance for the data. A value more far than 0.5 was considered as an indication of good performances (17). This technique reduces the number of observations by classifying them into homogeneous clusters, identifying the groups without previously knowing group memberships or the number of possible groups. Final groups were analyzed as dietary profiles and characterized with main synthetic statistics comparisons by univariate analysis.

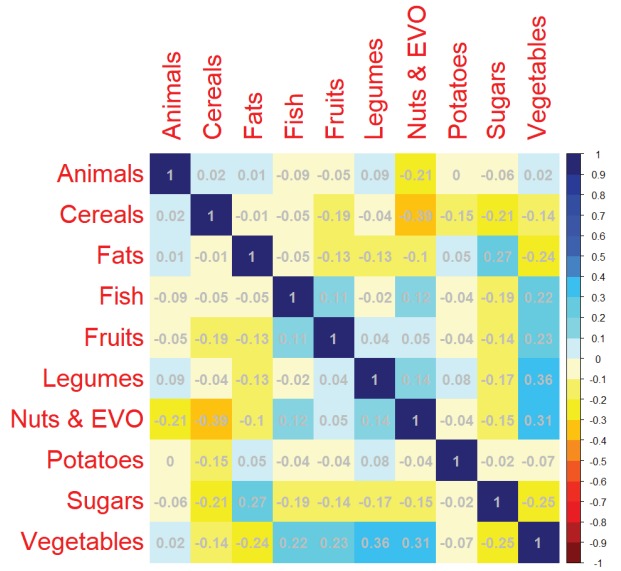

Figure 1. *Correlation matrix as heat-map of food groups.*

RESULTS

Patients' characteristics are reported in **Table 1**. Participants' mean age was 52 ± 9 years, BMI was 27.2 ± 5.8 kg/m^2, waist circumference was 94 ± 14 cm, 74% had low cancer stage (I or IIA), 65% had hormonal-dependent BC and reported medium adherence to the MD diet. **Table 2** shows patients' distribution according to BC molecular subtypes. No significant differences emerged between food groups and molecular subtypes. Pearson's correlation matrix was plotted in **Figure 2**. Statistically linear correlations were reported if the correlation coefficient ρ was greater than 0.3: vegetables with legumes (ρ = 0.36, p < 0.01) and with nuts & EVOO (ρ = 0.31, p < 0.01), nuts & EVOO with cereals (ρ = 0.39, p < 0.01). Overall PCA showed that the total variance explained 35.2% for the first two dimensions (**Figure 2**, whole data graph). The first factorial axis (or first PCA factor or dimension) was mainly built by vegetables (27.6%), nuts & EVOO (16.7%), and sweet & sugars (11.8%). Legumes and fats were just over

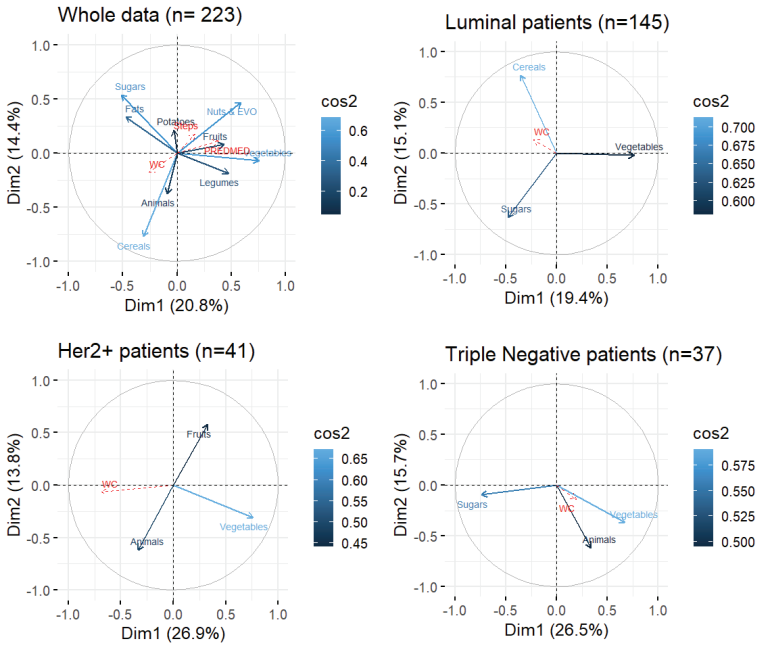

Figure 2. *Principal component analysis (PCA) results for the overall sample and by molecular subtypes.*

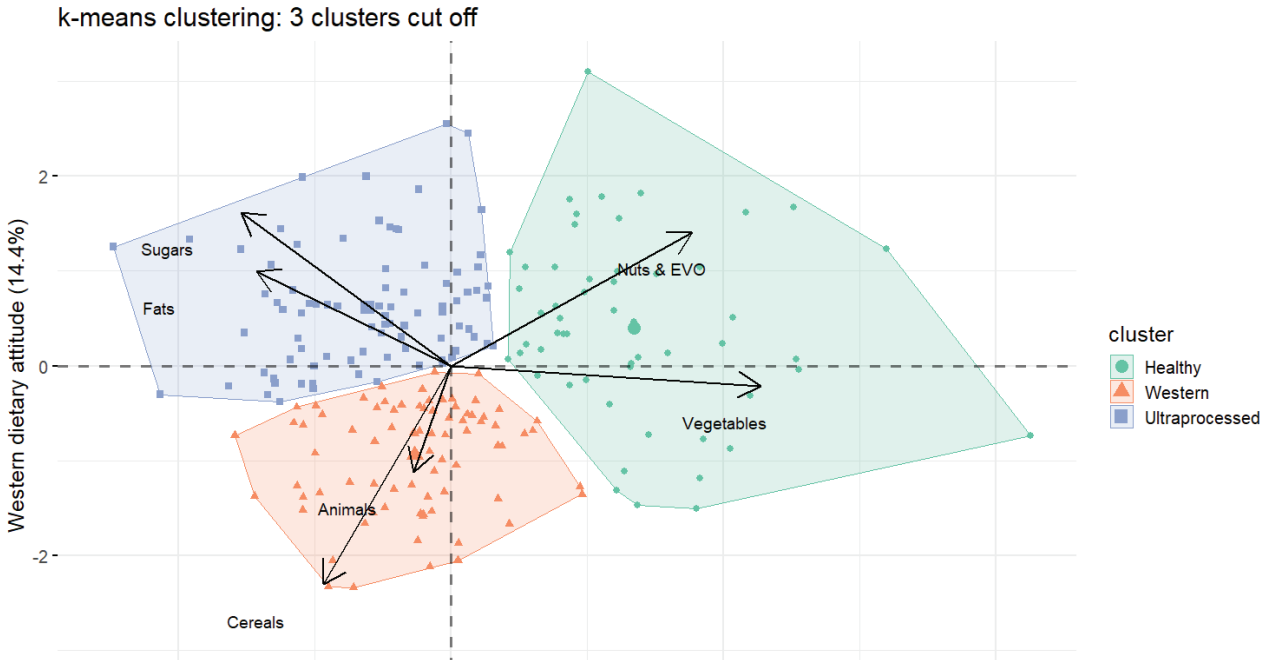

Figure 3. *K- means clusters in 2D-representation.*

10%. Similarly, cereals (40.9%), sugars & sweets (20.2%) and nuts & EVOO (15.4%) were main determinants of the second factorial axis. The third factorial axis was mainly composed of legumes (26.7%), potatoes (23.4%), animal protein (24.4%) and fish (16.7%). The fourth dimension was represented by fruits (34.5%), animal proteins (24.1%) and potatoes (19.3%), and the fifth dimension by potatoes (42.6%), fish (17.1%) and fruits (16.6%). In the analysis of molecular subtypes, the first and second dimensions explained >34% of the total variance. In the luminal subtype (**Figure 2**, Luminal graph) the main contribution was similar to that of the overall dataset. The first factorial axis was characterized mainly by vegetables (30.0%) and nuts & EVOO (17.5%); the second component by cere-

Table 2. *Distribution of patients' characteristics according to BC molecular subtypes.*

CHARACTERISTICS	LUMINAL N = 145[1]	HER2+ N = 41[1]	TRIPLE NEGATIVE N = 37[1]	P-VALUE[2]
Age (years)				0.803
Mean (SD)	52 (9)	52 (9)	51 (10)	
Cancer stage				0.203
IA-2A	108 (74%)	26 (63%)	32 (86%)	
2B	16 (11%)	6 (15%)	3 (8.1%)	
3A-3B	21 (14%)	9 (22%)	2 (5.4%)	
Waist circumference (cm)				0.636
Mean (SD)	93 (15)	95 (14)	94 (11)	
Body mass index, BMI (kg/m^2)				0.405
Mean (SD)	27.0 (5.9)	28.1 (6.1)	26.9 (5.4)	
BMI class (kg/m^2)				0.522
<25	61 (42%)	15 (37%)	19 (51%)	
25-29.9	49 (34%)	12 (29%)	9 (24%)	
≥30	35 (24%)	14 (34%)	9 (24%)	
Education (years)				0.915
Mean (SD)	12.6 (4.5)	12.6 (4.6)	12.9 (4.8)	
PREDIMED score (out of 14 points)				0.530
Mean (SD)	8.10 (2.01)	8.49 (1.57)	8.30 (1.94)	
Steps (n/day)				0.302
Mean (SD)	6,004 (2,856)	5,099 (2,514)	5,801 (2,700)	
Potatoes (grams/1000 Kcal/day)				0.459
Mean (SD)	13 (11)	15 (14)	16 (14)	
Fish (grams/1000 Kcal/day)				0.168
Mean (SD)	28 (21)	36 (28)	37 (31)	
Vegetables (grams/1000 Kcal/day)				0.242
Mean (SD)	124 (67)	140 (78)	146 (79)	
Fats and oil dressings (grams/1000 Kcal/day)				0.665
Mean (SD)	1.44 (1.95)	1.22 (1.42)	1.19 (1.50)	
Animal Proteins (grams/1000 Kcal/day)				0.354
Mean (SD)	50 (27)	46 (28)	48 (22)	
Cereals and Pizza (grams/1000 Kcal/day)				>0.999
Mean (SD)	109 (32)	111 (35)	110 (29)	
Legumes (grams/1000 Kcal/day)				0.593
Mean (SD)	18 (23)	22 (35)	17 (23)	
Fruits, fruit drinks and jams (grams/1000 Kcal/day)				0.209
Mean (SD)	219 (109)	243 (100)	233 (93)	
Sweets and sugars (grams/1000 Kcal/day)				0.805
Mean (SD)	32 (30)	33 (36)	33 (28)	
Nuts and EVOO (grams/1000 Kcal/day)				0.114
Mean (SD)	14 (10)	15 (7)	14 (6)	

[1]n (%); [2]Kruskal-Wallis rank sum test; Fisher's exact test; Pearson's Chi-squared test.

als (38.7%), sugars & sweets (26.3%), nuts & EVOO (15.9%) and fats (11.2%).

In the HER2+ subtype (n = 41) the first factorial axis was characterized mainly by nuts & EVOO (26.8%) and vegetables (22.4%), legumes (13.6%) and fish (11.7%) and the second factorial axis by animal proteins (27.6%), fruits (24.3%), legumes (18.5%) and fats (14.6%). Finally, in the TNBC subtype (n = 37) the strongest contribution to the first factorial axis was from sweet & sugars (20.7%), then vegetables (17.1%), fruits (11.9%) and legumes (11.0%) while animal proteins (24.3%), nuts & EVOO (16.0%), fruits (14.1%) and fish (12.1%) determined the second factor (**Figure 2**).

Three clusters of patients were found: cluster 1, Healthy (N = 54), cluster 2, Western (N = 79), and cluster 3 Ultra-processed (N = 85) (**Figure 3**). BC patients from cluster 1 (**Table 3**) were associated with healthier dietary habits compared to the other two groups, with approximately twice the vegetable intake compared with cluster 2 (204 grams *vs*. 119 grams for cluster 2 and 95 grams for cluster 3, $p < 0.01$). Cluster 1 also consumed more fish (44 grams *vs*. 29 grams and 24 grams, $p < 0.01$), fruits (296 grams *vs*. 208 grams and 201 grams, $p < 0.01$), legumes (34 grams *vs*. 18 grams and 10 grams, $p < 0.01$). BC patients from cluster 2 were more sedentary and with a higher BMI and central obesity (35% ≥30 kg/m^2 compared with 13% in cluster 1 and 25% in cluster 3, $p \leq 0.05$ for both comparisons), consumed more animal proteins (62 grams *vs.* 44 grams and 42 grams, $p < 0.01$) and cereals (135 grams *vs*. 91 for cluster 1 and 99 grams for cluster 3, $p < 0.01$ for both comparisons) the majority of which were refined (84%). BC patients belonging to cluster 1 were less overweight (25.2 kg/m^2 *vs*. 28.5 and 27.2, $p < 0.01$), with a higher adherence to the Mediterranean diet (PREDIMED score 9.2 *vs*. 7.9, out of 14) and with higher physical activity (8840 *vs.* 5121 steps/day for cluster 2 and 5796 steps/day for cluster 3, overall $p < 0.01$). Finally, cluster 3 consumed more than twice the amount of sweet & sugars (46 grams *vs*. 21 grams cluster 1 and 19 grams for cluster 2, $p < 0.01$) and four times the amount of fats compared with cluster 1 (2.0 grams *vs.* 0.5 grams and 1.0 grams, $p < 0.01$). BC molecular subtypes were equally distributed in cluster 1 and 3, while cluster 2 was mainly composed of luminal BC patients (72%) *vs*. 56% in cluster 1 and 64% in cluster 3, $p < 0.04$ for both while TNBC were little represented. HER2+ were distributed evenly among clusters (15-22%). Conversely, TNBC patients were found mostly in cluster 3 (51% of all TNBC) and cluster 1 (32% of all TNBC).

DISCUSSION

Our cluster analysis found three distinct dietary patterns: cluster 1-Healthy pattern characterized by vegetables, extra-virgin olive oil, tree nuts, fruits and legumes, cluster 2-Western pattern characterized by cereals and animal proteins and cluster 3-Ultraprocessed pattern characterized by sweets, sugars, and non-olive oil fats. The first cluster included more people with normal body weight and waist circumference. The second and third clusters included more people with higher body weight and waist circumference compared to cluster 1. Patients with hormone-related (luminal) BC tended to be older and were found mostly in cluster 2 while TNBC patients were found mostly in the Ultra-processed cluster 3. Considering that two thirds of our patients had hormone-related BC (luminal) it is discouraging to find that most women living in a Mediterranean country followed a Western dietary pattern. Moreover, the baseline diet is generally representative of patients' habitual diet suggesting a possible causal link with the development of this type of cancer. In a Spanish case-control study that used PCA analysis to derive three main dietary patterns, the Western dietary pattern, which is characterized by high intakes of refined cereals, animal sources of food, saturated fatty acids, and cholesterol, was associated with 46% increased risk in BC which increased to 75% in premenopausal women while the Mediterranean dietary pattern with a 44% protection (18). In this Mediterranean population the BC risk reduction with a Mediterranean diet was particularly strong in patients with TNBC (68%). This knowledge makes it even more relevant to strongly advise a Mediterranean diet to women with TNBC which is the most aggressive subtype and who tend to be younger women and high consumers of sweets and sugars and non-EVOO fats (ultra-processed pattern) in our study.

In the 2018 meta-analysis of international studies, the Mediterranean diet has been significantly and inversely associated with total mo rtality, cardiovascular and cancer mortality and with incidence of total cancer including BC (9). Case-control and cohort studies conducted in Italy after the 2018 meta-analysis indicate that a higher adherence to the Mediterranean diet was associated with 18% lower risk of BC (19) and with 63% higher 15-year surviv-

Table 3. *Distribution of main clusters.*

VARIABLE	CLUSTERS			P-VALUES	
	HEALTHY (CLUSTER 1) N = 54[1]	WESTERN (CLUSTER 2) N = 79[1]	ULTRA-PROCESSED (CLUSTER 3) N = 85[1]	CLUSTER 1 *VS.* CLUSTER 2[2]	CLUSTER 1 *VS.* CLUSTER 3[2]
Age (years)				0.781	0.162
Mean (SD)	52.4 (7.9)	52.6 (10.1)	51.4 (9.4)		
Education (years)				**0.020**	0.359
Mean (SD)	13.6 (3.9)	11.7 (4.6)	12.9 (5.0)		
Body mass index, BMI (kg/m^2)				**0.002**	**0.018**
Mean (SD)	25.2 (5.5)	28.5 (6.2)	27.2 (5.4)		
BMI class (kg/m^2)				**0.006**	0.051
<25	32 (59%)	28 (35%)	33 (39%)		
25-30	15 (28%)	23 (29%)	31 (36%)		
≥30	7 (13%)	28 (35%)	21 (25%)		
Waist circumference (cm)				**0.001**	**0.004**
Mean (SD)	87.9 (13.5)	96.7 (15.4)	94.2 (12.1)		
Waist circumference class (cm)				0.064	**0.033**
<88	28 (52%)	27 (34%)	27 (32%)		
≥88	26 (48%)	52 (66%)	57 (68%)		
PREDIMED score (out of 14 points)				**<0.001**	**<0.001**
Mean (SD)	9.2 (1.8)	7.9 (2.0)	7.9 (1.7)		
Steps (n/day)				**0.001**	0.057
Mean (SD)	6,840.1 (3,156.8)	5,121.5 (2,515.6)	5,796.0 (2,586.8)		
Molecular subtypes				**0.039**	0.448
Luminal	30 (56%)	57 (72%)	54 (64%)		
Her2+	12 (22%)	16 (20%)	12 (14%)		
Triple Negative	12 (22%)	6 (8%)	19 (22%)		
Animal proteins (meats and eggs, grams/1000 Kcal/day)				**<0.001**	0.794
Mean (SD)	43.9 (25.6)	61.5 (29.4)	42.0 (19.3)		
Cereals (grams/1000 Kcal/day)				**<0.001**	0.114
Mean (SD)	91.0 (27.3)	135.4 (23.0)	99.2 (26.7)		
Fats and oily dressings (grams/1000 Kcal/day)				**0.034**	**<0.001**
Mean (SD)	0.5 (0.6)	1.0 (1.3)	2.0 (2.0)		
Fish (grams/1000 Kcal/day)				**0.002**	**<0.001**
Mean (SD)	44.3 (28.9)	28.7 (23.5)	24.4 (18.7)		
Fruit, fruit drinks and jams (grams/1000Kcal/day)				**<0.001**	**<0.001**
Mean (SD)	296.2 (96.1)	207.8 (95.5)	200.9 (99.3)		
Median (IQR)	297.6 (224.2, 365.9)	202.7 (141.3, 270.1)	201.6 (129.1, 254.2)		
Legumes (grams/1000 Kcal/day)				**0.007**	**<0.001**
Mean (SD)	33.6 (39.5)	18.0 (21.2)	9.6 (8.5)		
Potatoes (grams/1000 Kcal/day)				0.658	0.468
Mean (SD)	13.6 (11.9)	12.0 (12.0)	15.5 (13.2)		
Nuts and EVOO (grams/1000 Kcal/day)				**<0.001**	**<0.001**
Mean (SD)	22.1 (8.8)	10.4 (4.1)	12.2 (5.7)		
Sweets and sugar (grams/1000 Kcal/day)				0.903	**<0.001**
Mean (SD)	20.5 (15.6)	19.3 (11.9)	45.9 (25.6)		
Vegetables (grams/1000 Kcal/day)				**<0.001**	**<0.001**
Mean (SD)	204.2 (77.3)	119.2 (50.3)	94.9 (46.5)		

[1]*n (%);* [2]*Wilcoxon rank sum test; Pearson's Chi-squared test.*

al (20). Oncologic treatment and side effects can be more challenging in women with overweight/obesity and metabolic diseases. Also, obesity is a known risk factor for BC, linked to excess adipose tissue which can increase circulating estrogen levels by higher aromatase activity, especially in the postmenopausal status, with consequent excessive hormonal stimulation to the mammary gland. However, interventions with the Mediterranean diet have been effective in reducing body weight (21) and cardiovascular events (22) in Mediterranean populations. Features of the Mediterranean diet (*i.e.*, high vegetable and fruit intakes) have shown beneficial effects on BC survival in the clinical trials WINS and WHEL conducted in the USA (23, 24). Furthermore, within the Mediterranean diet, a less glycemic dietary pattern that induces insulin economy, may contribute not only to lower diabetes risk but also BC risk: observational studies showed that a diabetes-risk reduction diet (DRRD) reduced BC risk by 24% in an Italian population (25) and reduced BC-specific mortality by 20% and overall mortality by 34% in an American population (26). A low glycemic index diet has shown to reduce BC risk by 6-8% in international studies (27, 28) and by 40% in an Italian population (29). Consuming a large amount of the daily caloric requirements in the form of sweets and refined carbohydrates may increase both the dietary glycemic index (GI) and the DRRD, however adhering to the traditional Mediterranean diet helps to reduce the dietary GI and the inflammatory potential of the diet (30). Furthermore, diets rich in ultra-processed foods which include processed meats, soft drinks, biscuits, and commercial sweets (our clusters 2 and 3), have been associated with higher risk of cardiometabolic diseases and cancer (31, 32). Ultra-processed foods represent 58% of the total calories consumed in a typical Western diet (33) although in our study sample it was 3-fold lower. The potential mechanisms of action of the Mediterranean diet, low GI diets, DRRD, and ultra-processed foods are several and could be distinguished into two main pathways: an oxidant/inflammation pathway and a glycemic/insulinemic pathway. A high intake of saturated fatty acids, such as red and processed meat, increase inflammatory processes and may generate more reactive oxygen species (ROS) which can damage cell membranes and increase DNA mutations (34). A diet rich in refined carbohydrates, sugars and sweets is characterized by a high GI index which may also increase inflammation, ROS and insulin levels. Insulin is an anabolic hormone with high homology for insulin-like growth factor 1 (IGF1), which stimulates cell proliferation (34). This type of diet was found directly associated with the risk of developing cancers at various sites (19) including BC (29). Foods with a protective role include: 1) whole grains, rich in fiber, phenolic compounds, minerals, vitamins and other trace elements, which in addition to increasing the sense of satiety, reduce the glycemic response and improve insulin sensitivity; 2) nuts, characterized by a rich mono/polyunsaturated fatty acid profile and by a high content of fiber and polyphenols, which contribute to the reduction of the risk of cardiovascular diseases and diabetes; 3) extra-virgin olive oil, characterized by a high content of polyphenols, mono-unsaturated fatty acids, vitamin E and chlorophyll which together concur to reduce cellular and DNA oxidation.

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CONCLUSIONS

Our findings revealed three main dietary risk group by BC patients at the baseline visit of a lifestyle Trial: a healthy dietary group (cluster 1), a western diet group (cluster 2) and an ultra-processed food diet group (cluster 3).

The largest European Epidemiologic Investigation on Diet and Cancer (EPIC) study showed that the consumption of fresh fruit and vegetables, especially green leafy vegetables (lettuce, spinach, chard) reduced the risk of developing BC (35). Part of the protective role of this type of dietary pattern comes from dietary fiber, vitamins, and minerals, flavonoids which act as antioxidants, immune enhancers, and hormone regulators.

A healthy diet, based on a Mediterranean dietary pattern and regular daily physical activity, as recommended by the international guidelines for the primary and secondary prevention of cancer (6), could improve the metabolic and hormonal profile, support immune function and limit inflammation thereby reducing the risk of BC and improving disease outcomes (6, 7).

COMPLIANCE WITH ETHICAL STANDARDS

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Conflict of interests

The Authors have declared no conflict of interests.
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Availability of data and materials

The data presented in this study are available at: https://doi.org/10.5281/zenodo.7688957;

Authors' contributions

CA, AL: conceptualization, writing, original draft preparation; CA, AL, CS: writing, review and editing; VS, PE, AL, MC, PG, LA: performing follow-up visits; CA, CS, DGP: performing statistical analyses; PM, PG, VS, PE, AL, PR: data managing; RE, GM, CE: research support; AL: language editing; IF, CM, DLM, AA: medical and surgical support; CEg, PF: management.

Ethical approval

Human studies and subjects

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Ethics Committee of each recruiting hospital (ClinicalTrials.gov NCT02786875; 17 March 2016). Informed Consent Statement: informed consent was obtained from all subjects involved in the study.

Animal studies

N/A.

Publications ethics

Plagiarism

The contents of the article are original and any overlaps with other articles are by the Authors themselves and appropriately cited.

Data falsification and fabrication

All the data correspond to the real.

REFERENCES

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-49. doi: 10.3322/caac.21660.
2. AIOM/AIRTUM, I NUMERI DEL CANCRO IN ITALIA. 2022.
3. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010;363(20):1938-48. doi: 10.1056/NEJMra1001389.
4. Lebert JM, Lester R, Powell E, Seal M, McCarthy J. Advances in the systemic treatment of triple-negative breast cancer. Curr Oncol. 2018;25(Suppl.1):S142-50. doi: 10.3747/co.25.3954.
5. Willett WC, Diet, nutrition, and avoidable cancer. Environ Health Perspect. 1995;103 (Suppl 8):165-70. doi: 10.1289/ehp.95103s8165.
6. World Cancer Research Fund/American Institute for Cancer Research. Diet, Nutrition, Physical Activity and Cancer: A Global Perspective. Continuous Update Project Expert Report 2018. Available from: Dietandcancerreport.org. Accessed: Oct 1, 2019.
7. Becerra-Tomás N, Balducci K, Abar L, Aune D, Cariolou M, Greenwood DC, et al. Postdiagnosis dietary factors, supplement use and breast cancer prognosis: Global Cancer Update Programme (CUP Global) systematic literature review and meta-analysis. Int J Cancer. 2023. 152(4):616-34. doi: 10.1002/ijc.34321.

8. Martínez-González MÁ, Hershey MS, Zazpe I, Trichopoulou A. Transferability of the Mediterranean Diet to Non-Mediterranean Countries. What Is and What Is Not the Mediterranean Diet. Nutrients. 2017;9(11):1226. doi: 10.3390/nu9111226. Erratum in: Nutrients. 2018 Jun 26;10(7).
9. Dinu M, Pagliai G, Casini A, Sofi F. Mediterranean diet and multiple health outcomes: an umbrella review of meta-analyses of observational studies and randomised trials. Eur J Clin Nutr. 2018;72(1):30-43. doi: 10.1038/ejcn.2017.58.
10. Panagiotakos DB, Pitsavos C, Stefanadis C. Dietary patterns: a Mediterranean diet score and its relation to clinical and biological markers of cardiovascular disease risk. Nutr Metab Cardiovasc Dis. 2006;16(8):559-68. doi: 10.1016/j.numecd.2005.08.006.
11. Schwingshackl L, Missbach B, Konig J, Hoffmann G. Adherence to a Mediterranean diet and risk of diabetes: a systematic review and meta-analysis. Public Health Nutr. 2015;18(7):1292-9. doi: 10.1017/S1368980014001542.
12. Turati F, Carioli G, Bravi F, Ferraroni M, Serraino D, Montella M, et al. Mediterranean Diet and Breast Cancer Risk. Nutrients. 2018;10(3):326. doi: 10.3390/nu10030326.
13. Toledo E, Salas-Salvadó J, Donat-Vargas C, Buil-Cosiales P, Estruch R, Ros E, et al. Mediterranean Diet and Invasive Breast Cancer Risk Among Women at High Cardiovascular Risk in the PREDIMED Trial: A Randomized Clinical Trial. JAMA Intern Med. 2015;175(11):1752-60. doi: 10.1001/jamainternmed.2015.4838. Erratum in: JAMA Intern Med. 2018;178(12):1731-2.
14. Farvid MS, Chen WY, Rosner BA, Tamimi RM, Willett WC, Eliassen AH. Fruit and vegetable consumption and breast cancer incidence: Repeated measures over 30 years of follow-up. Int J Cancer. 2019;144(7):1496-510. doi: 10.1002/ijc.31653.
15. Augustin LS, Libra M, Crispo A, Grimaldi M, De Laurentiis M, Rinaldo M, et al. Low glycemic index diet, exercise and vitamin D to reduce breast cancer recurrence (DEDiCa): design of a clinical trial. BMC Cancer. 2017;17(1):69. doi: 10.1186/s12885-017-3064-4.
16. Schröder H, Fitó M, Estruch R, Martínez-González MA, Corella D, Salas-Salvadó J, et al. A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women. J Nutr. 2011;141(6):1140-5. doi: 10.3945/jn.110.135566.
17. Lawson RG, Jurs PC. 1990. New Index for Clustering Tendency and Its Application to Chemical Problems. J Chem Inf Comput Sci. 1990;30(1):36-41. doi: 10.1021/ci00065a010.
18. Castelló A, Pollán M, Buijsse B, Ruiz A, Casas AM, Baena-Cañada JM, et al. Spanish Mediterranean diet and other dietary patterns and breast cancer risk: case–control EpiGEICAM study. Br J Cancer. 2014;111(7):1454-62. doi: 10.1038/bjc.2014.434.
19. Turati F, Galeone C, Augustin LSA, La Vecchia C. Glycemic Index, Glycemic Load and Cancer Risk: An Updated Meta-Analysis. Nutrients. 2019;11(10):2342. doi: 10.3390/nu11102342.
20. Di Maso M, Dal Maso L, Augustin LSA, Puppo A, Falcini F, Stocco C, et al. Adherence to the Mediterranean Diet and Mortality after Breast Cancer. Nutrients. 2020;12(12):3649. doi: 10.3390/nu12123649.
21. Mancini JG, Filion KB, Atallah R, Eisenberg MJ. Systematic Review of the Mediterranean Diet for Long-Term Weight Loss. Am J Med. 2016;129(4):407-15.e4. doi: 10.1016/j.amjmed.2015.11.028.
22. Estruch R, Ros E, Salas-Salvadó J, Covas MI, Corella D, Arós F, et al; PREDIMED Study Investigators. Primary Prevention of Cardiovascular Disease with a Mediterranean Diet Supplemented with Extra-Virgin Olive Oil or Nuts. N Engl J Med. 2018;378(25):e34. doi: 10.1056/NEJMoa1800389.
23. Chlebowski RT, Blackburn GL, Thomson CA, Nixon DW, Shapiro A, Hoy MK, et al. Dietary fat reduction and breast cancer outcome: interim efficacy results from the Women's Intervention Nutrition Study. J Natl Cancer Inst. 2006;98(24):1767-76. doi: 10.1093/jnci/djj494.
24. Pierce JP, Natarajan L, Caan BJ, Parker BA, Greenberg ER, Flatt SW, et al. Influence of a diet very high in vegetables, fruit, and fiber and low in fat on prognosis following treatment for breast cancer: the Women's Healthy Eating and Living (WHEL) randomized trial. JAMA. 2007;298(3):289-98. doi: 10.1001/jama.298.3.289.
25. Turati F, Bravi F, Rossi M, Serraino D, Mattioli V, Augustin L, et al. Diabetes risk reduction diet and the risk of breast cancer. Eur J Cancer Prev. 2022;31(4):339-45. doi: 10.1097/CEJ.0000000000000709.
26. Wang T, Farvid MS, Kang JH, Holmes MD, Rosner BA, Tamimi RM, et al. Diabetes Risk Reduction Diet and Survival after Breast Cancer Diagnosis. Cancer Res, 2021. 81(15):4155-62. doi: 10.1158/0008-5472.CAN-21-0256.

27. Choi Y, Giovannucci E, Lee JE. Glycaemic index and glycaemic load in relation to risk of diabetes-related cancers: a meta-analysis. Br J Nutr. 2012;108(11):1934-47. doi: 10.1017/S0007114512003984.
28. Turati F, Galeone C, Gandini S, Augustin LS, Jenkins DJ, Pelucchi C, et al. High glycemic index and glycemic load are associated with moderately increased cancer risk. Mol Nutr Food Res. 2015;59(7):1384-94. doi: 10.1002/mnfr.201400594.
29. Augustin LS, Dal Maso L, La Vecchia C, Parpinel M, Negri E, Vaccarella S, et al. Dietary glycemic index and glycemic load, and breast cancer risk: a case-control study. Ann Oncol. 2001;12(11):1533-8. doi: 10.1023/a:1013176129380.
30. Vitale S, Palumbo E, Polesel J, Hebert JR, Shivappa N, Montagnese C, et al. One-year nutrition counselling in the context of a Mediterranean diet reduced the dietary inflammatory index in women with breast cancer: a role for the dietary glycemic index. Food Funct. 2023;14(3):1560-72. doi: 10.1039/d2fo02198f.
31. Elizabeth L, Machado P, Zinöcker M, Baker P, Lawrence M. Ultra-Processed Foods and Health Outcomes: A Narrative Review. Nutrients. 2020;12(7):1955. doi: 10.3390/nu12071955.
32. Chang K, Gunter MJ, Rauber F, Levy RB, Huybrechts I, Kliemann N, et al. Ultra-processed food consumption, cancer risk and cancer mortality: a large-scale prospective analysis within the UK Biobank. EClinicalMedicine. 2023;56:101840. doi: 10.1016/j.eclinm.2023.101840.
33. Steele EM, Khandpur N, Sun Q, Monteiro CA. The impact of acculturation to the US environment on the dietary share of ultra-processed foods among US adults. Prev Med. 2020;141:106261. doi: 10.1016/j.ypmed.2020.106261.
34. Augustin LS, Franceschi S, Jenkins DJ, Kendall CW, La Vecchia C. Glycemic index in chronic disease: a review. Eur J Clin Nutr. 2002;56(11):1049-71. doi: 10.1038/sj.ejcn.1601454.
35. Buckland G, Travier N, Cottet V, Gonzalez CA, Lujan-Barroso L, Agudo A, et al. Adherence to the mediterranean diet and risk of breast cancer in the European prospective investigation into cancer and nutrition cohort study. Int J Cancer. 2013;132(12):2918-27. doi: 10.1002/ijc.27958.