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ORIGINAL PAPER

Post-diagnosis serum 25-hydroxyvitamin D concentrations in women treated for breast cancer participating in a lifestyle trial in Italy

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SUMMARY

Objective. To report cross-sectionally serum levels of 25-hydroxyvitamin D [25(OH)D] in women living in Italy within 12 months from breast cancer (BC) diagnosis.

Methods. Baseline data were obtained from 394 women diagnosed with primary BC, enrolled from 2016 to 2019 in a lifestyle trial conducted in Italy. Subjects' characteristics were compared between two 25(OH)D concentrations (hypovitaminosis D<20 and ≥20 ng/mL) with the Chi-squared test or Fisher's exact test for small-expected counts. Using multiple logistic regression-adjusted models, we estimated odds ratios (ORs) of hypovitaminosis D with 95% confidence intervals (CIs) in the total sample and the unsupplemented subgroup.

Results. Hypovitaminosis D was found in 39% of all subjects, 60% in unsupplemented subjects, and 10% in supplemented subjects. Increasing ORs of hypovitaminosis D were found with increasing body mass index, 25-30, >30, and ≥35 *versus* <25 kg/m^2 (ORs: 2.50, 4.64, and 5.81, respectively, in the total cohort and ORs: 2.68, 5.38, and 7.08 in the unsupplemented one); living in the most southern Italian region compared to the northern region (OR 2.50, 95%CI 1.22-5.13); and with hypertriglyceridemia (OR 2.46; 95%CI 1.16-5.22), chemotherapy history (OR 1.86, 95%CI 1.03-3.38), and inversely with anti-estrogenic therapy (OR 0.43, 95%CI 0.24-0.75) in the total sample.

Conclusions. Hypovitaminosis D in women recently diagnosed with BC and participating in a lifestyle trial in Italy was widespread and highest with obesity, hypertriglyceridemia, and chemotherapy use. Considering that hypovitaminosis D is a risk factor for lower efficacy of bone density treatments and BC mortality, our results suggest the need to promptly address and treat vitamin D deficiency.

Key words: *Vitamin D, cholecalciferol, supplementation, breast cancer, chemotherapy, obesity.*

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INTRODUCTION

Breast cancer (BC) accounts for 30% of all new cancer diagnoses in females and still represents the most common cancer among them (1). Despite significant increases in survival rates in the last decades, a relevant risk of recurrence and death persists (2), and BC still remains the second leading cause of death among women globally (3). There is, therefore, a need for the identification of modifiable risk factors to further improve survival and survivors' quality of life (4).

A possible favorable role of vitamin D in BC has been explored in the last few decades. In a meta-analysis of observational studies, Song *et al.* (5) found a dose-response relationship between serum 25-hydroxyvitamin D [25(OH)D] concentrations and BC risk, while no associations were found with dietary vitamin D intakes. Interestingly, women with more aggressive BC seemed to have lower concentrations of circulating 25(OH)D (6). In BC survivors, Friedman *et al.* (7) showed that vitamin D deficiency was highly prevalent and was associated with BC recurrence and decreased survival. A summary of the literature published in 2021 investigating circulating 25(OH)D concentrations in women with BC on survival outcomes found 57% increased overall survival and 44% increased BC-specific survival with higher concentrations (8). Conversely, in 2017, a systematic review and meta-analysis of trial data (9), specifically focusing on cancer-related outcomes, reported null findings failing to support the clinical benefits of vitamin D supplementation. However, the latter meta-analysis had various critical issues beyond the simple lack of statistical power and depended on the inclusion of short-term studies with very low doses of vitamin D, different dosing schedules, different geographical locations, and the lack of a sufficient proportion of subjects with vitamin D deficiency at baseline (10). Indeed, a 2019 meta-analysis of randomized controlled trials (RCTs) showed that vitamin D supplementation may reduce cancer mortality, especially when a daily regimen is adopted (11). Similar findings in 2020 have been explicitly observed in a secondary analysis of the VITAL randomized controlled trial (12). In the VITAL trial, daily supplementation with 2000 UI vitamin D (cholecalciferol) reduced the incidence of metastatic and fatal cancer, with a more pronounced benefit in individuals with normal body weight. These results have been confirmed by the most recent dose-response meta-analysis commissioned by the World Cancer Research Fund, which found a 6% relative risk reduction of BC-specific mortality per 10 nmol/L of 25(OH)D (13).

Furthermore, data show that a proportion of women with early BC treated with cancer therapies that induce greater bone resorption and deregulation of bone turnover (*e.g.*, aromatase inhibitors, glucocorticoids, chemotherapy, radiation therapy) have sub-optimal circulating concentrations of 25(OH)D, which could further increase their risk for bone loss and fractures (14-16). Chemotherapy, in particular, may reduce circulating 25(OH)D concentrations (17-21) through altered activities of hepatic drug-metabolizing enzymes due to the upregulation of *CYP3A4* as a defense mechanism from highly toxic drugs and through the avoidance of sunlight due to chemotherapy-induced photosensitivity (22). Geographical location has been hypothesized to affect vitamin D status with a direct relationship between sunshine and 25(OH)D levels; however, this may not be true for southern European countries, where the evidence suggests the opposite, possibly due to the avoidance of sun exposure during intense heat (23).

Given that circulating 25(OH)D concentrations among BC survivors are typically low, which may negatively impact patients' prognosis, and since knowledge of the correlates of normal concentrations among BC survivors is limited, we examined factors that may be associated with vitamin D deficiency or sufficiency among BC survivors participating in the DEDiCa trial (diet, exercise, and vitamin D in BC recurrence) (24).

MATERIALS AND METHODS

This is a cross-sectional analysis of data from the DEDiCa study, which is an ongo-

ing multicenter randomized controlled trial of the effectiveness of a treatment program including dietary modification, physical activity, and vitamin D supplementation on BC recurrence conducted in five cancer centers in Italy. The main goal of the DEDiCa study is to test the effect of a 33-month lifestyle program combining quarterly advice on a Mediterranean diet, exercise, and vitamin D supplementation at two concentrations of intensity on BC recurrence and disease-free survival. Women aged ≥30 and <75 years (n=506) diagnosed with primary histological confirmed BC (stages I-III) in the previous 12 months and willing to sign the consent form were enrolled in the study. Suitable participants were identified through the surgical lists of collaborating hospitals, contacted by telephone, and offered to learn more about the study during group information sessions. The protocol was approved by the Italian Ministry of Health, the Italian Medicine Agency, and the ethics boards of each recruiting hospital (ClinicalTrials.gov, NCT02786875). The full details of the study protocol have been published elsewhere (24). Inclusion criteria were: women with a primary diagnosis of histologically confirmed BC without metastases (stages I-III; T1 with Ki67 ≥30%, T2 and T3) within 12 months from diagnosis; age ≥30 and <75 years; patients able to comprehend and willing to sign the consent form, and able to adhere to the protocol, including scheduled clinic visits and assigned treatment. Exclusion criteria were: patients who did not possess the inclusion criteria for this study; patients with sarcoidosis or other granulomatous diseases or with hypercalcemia (Ca>11 mg/dL); patients with any previous or current concomitant malignant cancer; pregnant or lactating women; patients with acquired immunodeficiency syndrome diagnosis; patients with severe renal insufficiency; patients with kidney stones (nephrocalcinosis or nephrolithiasis); patients participating in other lifestyle clinical trials.

For all other information (type of surgery, concomitant therapies, *etc.*), the reader is invited to refer to our previous publications (4, 24). For the present analysis, data from 394 women with BC recruited from November 2016 to April 2019 were included. During the baseline visit, information was collected on vitamin D supplementation (starting and ending dates, dose, and brand) prior to randomization, pharmacological therapies, smoking status, education, physical activity, anthropometric variables, blood pressure, serum 25(OH)D concentrations, and other biochemical analyses. The 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) as the coordinating center; *Clinica Mediterranea* (Naples); Villa Betania (Naples); Cannizzaro Hospital (Catania); San Vincenzo Hospital (Taormina); *Istituto Nazionale Tumori IRCCS CRO* (Aviano).

The multicenter nature of the study gave the opportunity to study vitamin D status among Italian regions (Friuli Venezia Giulia, Campania, Sicily) located at different latitudes (northern Italy, 46.2259°, center-south, 41.109947°, and far south, 37.500000°, respectively).

Biochemical analyses

Serum concentrations of 25(OH)D were analyzed using DiaSorin kits on the Liaison XL analyzer (DiaSorin, Saluggia, Italy) by the chemiluminescent method (CLIA). Serum concentrations of glucose, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and liver enzymes [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] were measured using reagents and an analyzer (Cobas C6000) by Roche Diagnostics (Monza, Italy) according to the manufacturer's instructions.

Serum samples collected in Vacutainer tubes without anticoagulant (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) were analyzed within 2 hours from a blood test or thawing (for samples shipped from distant recruiting centers). All analytes were measured in the coordinating hospital's routine analytical laboratory, undergoing standard quality control procedures.

Hypovitaminosis D, or vitamin D defi-

ciency for the current analysis, was defined as serum 25(OH)D concentrations <20 ng/mL. Insulin resistance was defined as a homeostatic model assessment for insulin resistance >2.

Statistical analysis

Categorical variables were compared between two baseline 25(OH)D concentrations (<20 and ≥20 ng/mL) using the Chi-squared test (or Fisher's exact test when appropriate, *i.e.*, small expected counts) in vitamin D orally supplemented patients and in non-supplemented patients. Supplemented patients were those who reported actively taking any dose of oral vitamin D before randomization.

Using adjusted multiple logistic regression models, we estimated the odds ratios (ORs) for vitamin D deficiency 25(OH)D<20 ng/mL) and their corresponding 95% confidence intervals (CIs) for the following variables: age (<45/45-49/50-54/55-59/60-64/≥65 years), geographical region (Campania/Sicily/Friuli Venezia Giulia), education (primary, middle/high school and university), body mass index (BMI) (<25/25-30/30-35/≥35 kg/m^2), steps/day (sedentary, low-active <7500 steps/day, medium and high-active ≥7500 steps/day) and smoking habits (never/ever/former). The same model, excluding the term for BMI, was used to estimate the OR for waist circumference (≤88/88-100.5/>100.5 cm). We calculated the ORs for vitamin D deficiency also for hypertriglyceridemia (<150/≥150 mg/dL), hypercholesterolemia (<130/≥130 mg/dL), AST (<32/≥32 U/L), ALT (<33/≥33 U/L), chemotherapy (yes/no), radiotherapy (yes/no), hormone therapy (yes/no) using multiple logistic regression models. These latter included adjustments for age, geographical region, education, BMI, steps/day, smoking habit, chemotherapy, seasonality of blood collection (April-October *versus* November-March) and oral vitamin D supplementation (yes/no) prior to randomization.

We conducted analyses in the total cohort of patients (n=394) as well as in the subgroup of women reporting no supplementation with oral vitamin D before randomization (unsupplemented n=227, 58% of the total). Statistical analyses were performed using SAS 9.4 (SAS Institute, Inc., Cary, NC, USA) and SPSS software, version 22 (SPSS, Inc., Chicago, IL, USA).

RESULTS

This cross-sectional analysis involved 394 women with BC. Baseline data on vitamin D supplementation prior to study start, serum of 25(OH)D concentrations, and other baseline characteristics are reported in Table I. Women with hypovitaminosis D represented 39% of the overall study population, 60% of the unsupplemented subgroup (n=227), and 10% of the supplemented subgroup (n=167). Women in the latter group reported oral cholecalciferol supplementation daily, weekly, bimonthly, or monthly before baseline at a corresponding daily dose of <1000 IU (n=27), 1000-2500 IU (n=52), 3000-4000 IU (n=76), 5000-10000 IU (n=7) while no specific dose was reported by five subjects. The average supplementation time was 15.3 months, with a median of 5.6 months (range: 4 days to 19 years before baseline). The univariate analysis performed in the unsupplemented subjects found a significant association between the prevalence of hypovitaminosis D and region of residence (geographical region), level of education, BMI, waist circumference, and daily steps.

Table II shows the adjusted OR of hypovitaminosis D with 95% CIs for hypertriglyceridemia, hypercholesterolemia, liver enzymes, and cancer therapies and selected characteristics in non-supplemented patients and in the total cohort. Patients living in Sicily were found to be at higher risk of hypovitaminosis D when compared to those living in Campania. This was observed both in the unsupplemented subgroup (OR: 2.40; 95% CI: 1.09-5.29) and in the whole study population (OR: 2.50; 95% CI: 1.22-5.13). Increasing ORs of hypovitaminosis D were found with increasing BMI (OR 2.5; 95% CI: 1.30-4.84; OR: 4.64; 95% CI: 2.08-10.35; OR: 5.81; 95% CI: 2.35-14.34 for BMI 25-29.9, 30-34.9, and ≥35, respectively, as compared to BMI<25) in the total sample. Similar results were seen

in the unsupplemented subgroup. These results were also reflected in classes of increasing waist circumference, where the ORs were 2-fold and 4-fold greater for waist circumferences of 88-100.5 cm and >100.5 cm, respectively. Hypertriglyceridemia (triglycerides>150 mg/dl) was associated with an over 2-fold higher risk of hypovitaminosis D, but only when considering the whole population (OR: 2.46; 95% CI: 1.16-5.22). Among cancer treatments, only chemotherapy was associated with hypovitaminosis D in the total cohort (OR: 1.86; 95% CI: 1.03-3.38). Interestingly, hypovitaminosis D was inversely related to oncologic hormonal therapy in the total sample (OR 0.43, 95% CI 0.24-0.75) and in the unsupplemented subgroup (OR: 0.53; 95% CI: 0.28-1.01). The results did not change after allowing for the season of

Table I - Distribution of 394 cases of breast cancer according to any oral vitamin D supplementation before the study start, baseline serum 25-hydroxyvitamin D concentrations and selected characteristics.

	Oral vitamin D supplements																		
	Yes					No					All						Total		
	Baseline 25(OH)D					Baseline 25(OH)D					Baseline 25(OH)D								
	<20 ng/mL		≥20 ng/mL			<20 ng/mL		≥20 ng/mL			<20 ng/mL		≥20 ng/mL						
	n	%	n	%	Media±DS	n	%	n	%	Media±DS	n	%	n	%	Media±DS	Media±DS	n	%	Media±DS
Total	17	100	150	100		136	100	91	100		153		241				394	100	
Age					53.5±9.9					52.6±8.7					53.8±8.8	52.4±9.4			52.9±9.2
<45	3	17.6	29	19.3		20	14.7	21	23.1		23	15.0	50	20.8			73	18.5	
45-49	3	17.6	29	19.3		32	23.5	22	24.2		35	22.9	51	21.2			86	21.8	
50-54	3	17.6	29	19.3		27	19.9	18	19.8		30	19.6	47	19.5			77	19.5	
55-59	1	5.9	23	15.3		25	18.4	16	17.6		26	17.0	39	16.2			65	16.5	
60-64	2	11.8	17	11.3		12	8.8	8	8.8		14	9.2	25	10.4			39	9.9	
≥65	5	29.4	23	15.3		20	14.7	6	6.6		25	16.3	29	12.0			54	13.7	
p value[a]	0.73					0.38					0.67								
Geographical area																			
Campania	10	58.8	120	80.0		77	56.6	63	69.2		87	56.9	183	75.9			270	68.5	
Sicily	1	5.9	6	4.0		43	31.6	14	15.4		44	28.8	20	8.3			64	16.2	
Friuli Venezia Giulia	6	35.3	24	16.0		16	11.8	14	15.4		22	14.4	38	15.8			60	15.2	
p value[a]	0.12					0.022					<0.001								
Education[b]																			
Primary and Middle school	6	37.5	47	32.4		58	45.0	20	23.3		64	44.1	67	29.0			131	34.8	
High school and University	10	62.5	98	67.6		71	55.0	66	76.7		81	55.9	164	71.0			245	65.2	
p value[a]	0.68					0.001					0.003								
Smoking habit[b]																			
Never	10	58.8	76	51.0		58	43.0	44	51.2		68	44.8	120	51.1			188	48.6	
Ever	3	17.6	30	20.1		26	19.3	14	16.3		29	19.1	44	18.7			73	18.9	
Former	4	23.5	43	28.9		51	37.8	28	32.6		55	36.2	71	30.2			126	32.6	
p value[a]	0.83					0.49					0.41								

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	Oral vitamin D supplements																		
	Yes					No					All						Total		
	Baseline 25(OH)D					Baseline 25(OH)D					Baseline 25(OH)D								
	<20 ng/mL		≥20 ng/mL			<20 ng/mL		≥20 ng/mL			<20 ng/mL		≥20 ng/mL						
	n	%	n	%	Media±DS	n	%	n	%	Media±DS	n	%	n	%	Media±DS	Media±DS	n	%	Media±DS
BMI (kg/m²)[b]					27.8±5.9					27.9±6.0					29.4±5.9	26.8±5.7			27.8±5.9
<25	4	23.5	62	41.6		34	25.2	49	55.1		38	25.0	111	46.6			149	38.2	
25-30	4	23.5	40	26.8		47	34.8	26	29.2		51	33.6	66	27.7			117	30.0	
30-35	5	29.4	28	18.8		28	20.7	9	10.1		33	21.7	37	15.6			70	17.9	
≥35	4	23.5	19	12.8		26	19.3	5	5.6		30	19.7	24	10.1			54	13.8	
p value[a]	0.33					<0.0001					0.0001								
Waist circumference (cm)[b]					95.3±14.5					95.7±14.3					99.2±13.6	93.2±14.3			95.5±14.3
≤88	3	17.6	55	36.9		30	22.2	43	48.3		33	21.7	98	41.2			131	33.6	
88-100,5	5	29.4	46	30.9		52	38.5	27	30.3		57	37.5	73	30.7			130	33.3	
>100,5	9	52.9	48	32.2		53	39.3	19	21.3		62	40.8	67	28.2			129	33.1	
p value[a]	0.17					0.0002													
Steps/day[b]					5681.0±2755					5786.3±2822					5150.2±2535.2	6125.3±2884.6			5741.3±2790
Sedentary <5000	8	47.1	69	46.6		68	50.4	31	36.0		76	50.0	100	42.7			176	45.6	
Low active 5000-7499	6	35.3	48	32.4		42	31.1	25	29.1		48	31.6	73	31.2			121	31.3	
Medium and high active ≥7500	3	17.6	31	20.9		25	18.5	30	34.9		28	18.4	61	26.1			89	23.1	
p value[a]	0.94					0.017					0.18								
AST (U/L)					19.2±7.2					20.1±8.3					20.8±9.1	19.1±6.9	378	95.9	19.7±7.9
<32	15	88.2	146	97.3		128	94.1	89	97.8		143	93.5	235	97.5					
≥32	2	11.8	4	2.7		8	5.9	2	2.2		10	6.5	6	2.5			16	4.1	
p value[a]	0.11					0.32					0.047								
ALT (U/L)					19.8±11.9					22.5±15.7					24.1±17.3	19.6±11.6	347	88.1	21.4±14.2
<33	15	88.2	138	92.0		112	82.4	82	90.1		127	83.0	220	91.3					
≥33	2	11.8	12	8.0		24	17.6	9	9.9		26	17.0	21	8.7			47	11.9	
p value[a]	0.64					0.1					0.014								
Hyper-triglyceridemia (mg/dL)					104.4±53.3					1078±58.0					120.5±70.5	97.4±42.2			106.36±56.0
<150	10	58.8	132	88.0		109	80.1	81	89.0		119	77.8	213	88.4			332	84.3	
≥150	7	41.2	18	12.0		27	19.9	10	11.0		34	22.2	28	11.6			62	15.7	
p value[a]	0.005					0.08					0.005								
Hyper-cholesterolemia[b] *(mg/dL)*					123.8±32.8					128.0±34.6					133.8±34.8	121.3±32.5			126.2±33.9
<130	5	29.4	93	62.0		74	54.8	57	62.6		79	52.0	150	62.2			229	58.3	
≥130	12	70.6	57	38.0		61	45.2	34	37.4		73	48.0	91	37.8			164	41.7	
p value[a]	0.01					0.24					0.044								

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Oral vitamin D supplements																			
	Yes					No					All						Total		
	Baseline 25(OH)D					Baseline 25(OH)D					Baseline 25(OH)D								
	<20 ng/mL		≥20 ng/mL			<20 ng/mL		≥20 ng/mL			<20 ng/mL		≥20 ng/mL						
	n	%	n	%	Media±DS	n	%	n	%	Media±DS	n	%	n	%	Media±DS	Media±DS	n	%	Media±DS
Hyperglycemia[b] (mg/dL)																			
No	16	94.1	141	94.0		127	94.1	85	95.5		143	94.1	226	94.6			369	94.4	
Yes	1	5.9	9	6.0		8	5.9	4	4.5		9	5.9	13	5.4			22	5.6	
p value[a]	0.98					0.64					0.84								
Chemotherapy[b]																			
No	5	29.4	77	51.3		27	20.0	23	25.8		32	21.1	100	41.8			132	33.8	
Yes	12	70.6	73	48.7		108	80.0	66	74.2		120	79.0	139	58.2			259	66.2	
p value[a]	0.09					0.3					<0.0001								
Radiotherapy[b]																			
No	4	25.0	44	29.3		61	45.2	35	39.3		65	43.1	79	33.1			144	36.9	
Yes	12	75.0	106	70.7		74	54.8	54	60.7		86	57.0	160	67.0			246	63.1	
p value[a]	0.72					0.39					0.046								
Hormone-therapy[b]																			
No	8	50.0	27	18.2		75	55.6	39	44.3		83	55.0	66	28.0			149	38.5	
Yes	8	50.0	121	81.8		60	44.4	49	55.7		68	45.0	170	72.0			238	61.5	
p value[a]	0.003					0.1					<0.0001								

SD, standard deviation; 25(OH)D, 25-hydroxyvitamin D; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; [a]categorical variables were compared between groups using Chi-squared test or Fisher's Exact Test (for small expected counts only); [b]the sum does not add up to the total because of missing values.

blood drawing as a possible confounding variable in the model, except for the liver enzyme AST, which was no longer significantly associated with hypovitaminosis D (OR: 3.95; 95% CI: 0.95-16.49).

Table III reports the proportions of supplemented subjects and subjects with vitamin D deficiency, with and without oral vitamin D supplementation before baseline, in different geographical regions. The lowest percentage of people supplemented and the highest prevalence of hypovitaminosis D were found in Sicily. A significantly higher OR for vitamin D deficiency was found in patients with insulin resistance when evaluated by the homeostatic model assessment index (OR: 2.16; 95% CI: 1.24-3.75, p=0.0063), but significance was lost after correction for multiple confounding factors such as BMI and waist circumference.

Among the supplemented subjects, 10% presented with hypovitaminosis D [25(OH)D <20 ng/mL], although these women also had a higher mean BMI of 30.5±5.9 compared to 27.5±5.8 kg/m^2 in people without hypovitaminosis D (p=0.046).

DISCUSSION

We investigated participants' characteristics associated with hypovitaminosis D in women enrolled in the DEDiCa trial (24), an ongoing multicenter RCT evaluating the effectiveness of a treatment program inclusive of dietary modification, physical activity, and vitamin D supplementation on BC recurrence in women with BC. Our results from baseline data confirm that hypovitaminosis D in Italy is common in BC patients, regardless of latitude and age. The

Table II - Distribution of breast cancer cases by serum 25-hydroxyvitamin D concentrations and odds ratios with 95% confidence intervals for selected characteristics in non-vitamin D supplemented patients before the study start and in the total sample.

	Non-vitamin D supplemented (227)			Total sample (394)			
	25(OH)D (<20 ng/mL) n (%)	25(OH)D (≥20 ng/mL) n (%)	OR (95% CI)	25(OH)D (<20 ng/mL) n (%)	25(OH)D (≥20 ng/mL) n (%)	OR[a] (95% CI)	OR[b] (95% CI)
Geographical region							
Campania	77 (56.6)	63 (69.2)	1[c]	87 (56.9)	183 (75.9)	1[c]	1[c]
Sicily	43 (31.6)	14 (15.4)	2.40 (1.09-5.29)	44 (28.8)	20 (8.3)	2.50 (1.22-5.13)	2.86 (1.36-6.02)
Friuli Venezia Giulia	16 (11.8)	14 (15.4)	1.30 (0.53-3.22)	22 (14.4)	38 (15.8)	2.02 (0.95-4.33)	1.95 (0.90-4.19)
Education							
High school and university	71 (55)	66 (76.7)	1[c]	81 (55.9)	164 (71)	1[c]	1[c]
Primary and middle school	58 (45)	20 (23.3)	1.81 (0.92-3.56)	64 (44.1)	67 (29)	1.62 (0.92-2.87)	1.60 (0.89-2.88)
Age (years)							
<45	20 (14.8)	21 (23.1)	1[c]	23 (15)	50 (20.8)	1[c]	1[c]
45-49	32 (23.5)	22 (24.2)	1.92 (0.75-4.86)	35 (22.9)	51 (21.2)	1.46 (0.64-3.31)	1.66 (0.72-3.85)
50-54	27 (19.9)	18 (19.8)	1.47 (0.56-3.87)	30 (19.6)	47 (19.5)	1.19 (0.51-2.784)	1.26 (0.53-3.02)
55-59	25 (18.4)	16 (17.6)	1.75 (0.64-4.82)	26 (17)	39 (16.2)	1.18 (0.49-2.83)	1.38 (0.56-3.39)
60-64	12 (8.8)	8 (8.8)	0.63 (0.17-2.27)	14 (9.2)	25 (10.4)	0.70 (0.23-2.14)	0.77 (0.25-2.41)
≥65	20 (14.7)	6 (6.6)	2.06 (0.59-7.21)	25 (16.3)	29 (12)	1.91 (0.71-5.14)	2.29 (0.83-6.32)
BMI (kg/m²)							
<25	34 (25.2)	49 (55.1)	1[c]	38 (25.0)	111 (46.6)	1[c]	1[c]
25-30	47 (34.8)	26 (29.2)	2.68 (1.28-5.63)	51 (33.6)	66 (27.7)	2.50 (1.30-4.84)	2.52 (1.29-4.91)
30-35	28 (20.7)	9 (10.1)	5.38 (2.01-14.40)	33 (21.7)	37 (15.6)	4.64 (2.08-10.35)	4.62 (2.05-10.43)
≥35	26 (19.3)	5 (5.6)	7.08 (2.18-22.97)	30 (19.7)	24 (10.1)	5.81 (2.35-14.34)	5.95 (2.38-14.90)
Waist circumference (cm)							
≤88	30 (22.2)	43 (48.3)	1[c]	33 (21.7)	98 (41.2)	1[c]	1[c]
88-100.5	52 (38.5)	27 (30.3)	2.62 (1.27-5.43)	57 (37.5)	73 (30.7)	2.69 (1.40-5.17)	2.49 (1.28-4.83)
>100.5	53 (39.3)	19 (21.4)	4.18 (1.85-9.47)	62 (40.8)	67 (28.2)	4.06 (2.02-8.17)	3.91 (1.93-7.91)
Steps/day							
Medium and high active ≥7500	25 (18.5)	30 (34.9)	1[c]	28 (18.4)	61 (26.1)	1[c]	1[c]
Low active 5000-7499	42 (31.1)	25 (29.1)	2.15 (0.95-4.85)	48 (31.6)	73 (31.2)	1.90 (0.93-3.88)	1.66 (0.80-3.45)
Sedentary <5000	68 (50.4)	31 (36.1)	1.75 (0.78-3.95)	76 (50.0)	100 (42.7)	1.68 (0.82-3.45)	1.33 (0.64-2.78)
Hypercholesterolemia (mg/dL)							
<150	109 (80.2)	81 (89)	1[c]	119 (77.8)	213 (88.4)	1[c]	1[c]
≥150	27 (19.9)	10 (11)	1.55 (0.63-3.83)	34 (22.2)	28 (11.6)	2.46 (1.16-5.22)	2.45 (1.13-5.29)
Colesterolemia (mg/dL)							
<130	74 (54.8)	57 (62.6)	1[c]	79 (51)	150 (62.2)	1[c]	1[c]
≥130	61 (45.2)	34 (37.4)	1.30 (0.69-2.44)	73 (48)	91 (37.8)	1.49 (0.87-2.55)	1.50 (0.86-2.59)

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	Non-vitamin D supplemented (227)			Total sample (394)			
	25(OH)D (<20 ng/mL) n (%)	25(OH)D (≥20 ng/mL) n (%)	OR (95% CI)	25(OH)D (<20 ng/mL) n (%)	25(OH)D (≥20 ng/mL) n (%)	OR[a] (95% CI)	OR[b] (95% CI)
AST (U/L)							
<32	128 (94.1)	89 (97.8)	1[c]	143 (93.5)	235 (97.5)	1[c]	1[c]
≥32	8 (5.9)	2 (2.2)	3.05 (0.53-17.36)	10 (6.5)	6 (2.5)	4.86 (1.17-20.10)	3.95 (0.94-16.49)
ALT[d] (U/L)							
<33	112 (82.4)	82 (90.1)	1[c]	127 (83)	220 (91.3)	1[c]	1[c]
≥33	24 (17.7)	9 (9.9)	1.75 (0.68-4.49)	26 (17)	21 (8.7)	1.66 (0.74-3.72)	1.56 (0.69-3.51)
Chemotherapy							
No	27 (20)	23 (25.8)	1[c]	32 (21.1)	100 (41.8)	1[c]	1[c]
Yes	108 (80)	66 (74.2)	1.49 (0.72-3.12)	120 (79)	139 (58.2)	1.86 (1.03-3.38)	1.87 (1.02-3.42)
Hormonotherapy[d]							
No	75 (55.6)	39 (44.3)	1[c]	83 (55)	66 (28)	1[c]	1[c]
Yes	60 (44.4)	49 (55.7)	0.53 (0.28-1.01)	68 (45)	170 (72)	0.43 (0.24-0.75)	0.47 (0.27-0.85)
Radiotherapy							
No	61 (45.2)	35 (39.3)	1[c]	65 (43.1)	79 (33.1)	1[c]	1[c]
Yes	74 (54.8)	54 (60.7)	0.80 (0.43-1.49)	86 (57)	160 (67)	0.90 (0.52-1.54)	0.86 (0.49-1.49)

25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; CI, confidence interval; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; [a]model adjusted for: age, geographical region, education, body mass index, steps/day and smoking habits; [b]model with additional seasonal adjustment April-October *versus* November-March; [c]reference category: aromatase inhibitors (n=139), luteinizing hormone-releasing hormone (n=52), tamoxifen (n=45).

higher prevalence of hypovitaminosis D was associated with overweight, measured either with BMI or waist circumference, and geographical region of residence.

We found a high prevalence of hypovitaminosis D (60%) in patients without any supplementation treatment, while a low prevalence (10%) was found in women who were treated with vitamin D supplementation at any dose prior to the study start. Similarly, the COBRA study in the Netherlands found 50% hypovitaminosis D in women with BC just before and during chemotherapy (17). The current recommendations for the correction of deficiency from the Italian Society of Osteoporosis and Bone Metabolic Diseases (25) suggest a total cumulative dose of 600,000-900,000 IU to be administered over a few weeks, followed by a maintenance dose of 800-2000 IU. According to the Summary of Product Characteristics of cholecalciferol, the highest dose allowed for the correction of vitamin D deficiency is 4000 IU daily,

Table III - The proportion of subjects with vitamin D deficiency (25-hydroxyvitamin D<20 ng/mL) by oral vitamin D supplementation before the study start, overall and by geographical region (north: Friuli Venezia Giulia; center-south: Campania; south: Sicily).

	All subjects (n=394)	Friuli Venezia Giulia (n=60)	Campania (n=270)	Sicily (n=64)
Supplemented prior to study start	167/394 (42.4%)	30/60 (50%)	130/270 (48.1%)	7/64 (10.9%)[a,b]
Vitamin D deficient	153/394 (38.8%)	22/60 (36.7%)	87/270 (32.2%)	44/64 (68.7%)[a,b]
Vitamin D deficient despite supplementation	17/167 (10.2%)	6/30 (20%)	10/130 (7.7%)	1/7 (14.3%)
Vitamin D deficiency non receiving supplementation	136/227 (59.9%)	16/30 (53.3%)	77/140 (55.0%)	43/57 (75.4%)[c,b]

[a]p<0.01 *versus* Friuli Venezia Giulia; [b]p<0.01 *versus* Campania; [c]p<0.05 *versus* Friuli Venezia Giulia.

followed by 750-2000 daily IU for maintenance (26).

The results of our study show that hypovitaminosis D increases with increasing BMI and waist circumference, not only in the unsupplemented subgroup but also in the whole sample studied. These data support the hypothesis of a potentially bi-directional relationship between hypovitaminosis D and obesity and waist circumference, already suggested by previous studies (27-30). These observations are of interest in light of recent data confirming the direct associations between obesity, high waist circumference, and cancer of the colon, corpus uteri, and postmenopausal BC (31). For this reason, it cannot be excluded that the association between vitamin D deficiency and BC could also be mediated by excess adiposity.

Aromatase inhibitors (AI) provide a significant benefit to BC patients with estrogen receptor positivity (32). However, they also modify body composition by increasing adiposity and the risk of osteoporosis and fractures (33). Thus, if high BMI and waist circumference increase the risk of BC and hypovitaminosis D, the use of AI could represent an additional risk factor for both fractures and vitamin D deficiency. Surprisingly, we observed a lower risk of hypovitaminosis D in patients taking anti-estrogenic therapy, even in the unsupplemented patients, albeit less strongly. If this is not a spurious finding related to more vitamin D supplementation associated with this class of medications, it may represent a beneficial side effect of anti-estrogenic therapy, perhaps due to the well-known interactions between vitamin D and sex steroids in oncology patients (34-36). This hypothesis, however, requires further confirmation in a study with a controlled vitamin D treatment and with repeated measurements of serum 25(OH)D and sex steroids over time.

The association between hypovitaminosis D and obesity-induced metabolic disorders is well established, although the mechanisms underlying these relationships have not been entirely elucidated (37). Calcitriol [1,25$(OH)_2$D)] may affect insulin secretion through the regulation of calcium fluxes into pancreatic beta-cells, insulin sensitivity by influencing the expression of insulin receptors through activation of the peroxisome proliferator activator receptor enzyme-delta, and beta-cell function by reducing cytokine-induced apoptosis (38-44). In the present study, we found a higher OR for vitamin D deficiency in patients with insulin resistance; however, significance was lost after correction for BMI, waist circumference, and physical activity. We suggest a possible sequestration of vitamin D by adipose tissue; however, due to our small sample size, this hypothesis requires further confirmation in larger studies.

When analyzing the region of residence, subjects living in Sicily (the southernmost location among DEDiCa recruiting centers) were at higher risk of hypovitaminosis D. In addition, this was the subgroup with the lowest rate of vitamin D supplementation (10% in Sicily *versus* 50% in Campania and Friuli Venezia Giulia), and as expected, supplemented subjects showed the lowest prevalence of hypovitaminosis D. It is reasonable to suspect that, in these regions, relying only on hypothetically "good" solar exposure might be associated with inadequate supplementation. These results support the consolidated findings that the southern European countries have the highest prevalence of vitamin D deficiency at all ages (23). In addition, the percentage of supplemented people was substantially similar at all ages, possibly suggesting that this aspect of general health is still poorly managed by both patients and clinicians. Of note, one supplemented subject out of 10 remained deficient despite vitamin D supplementation; this subgroup was also characterized by a higher BMI, confirming the role of fat mass in vitamin D status.

Age was not found to be a significant risk factor for hypovitaminosis D, and in all age groups, the prevalence of deficiency was close to or even higher than 50%. However, previous data in the general population showed an inverse relationship between age and vitamin D (45). This unexpected inconsistency may be a specific feature re-

lated to the disease itself or its treatments on vitamin D metabolism or a possible influence of vitamin D deficiency on oncogenesis (46).

When we considered the impact of the different pharmacological therapies in the whole cohort, we found that chemotherapy was associated with a significant increase in the risk of hypovitaminosis D. In our opinion, this finding may represent a reason in favor of cholecalciferol supplementation, even if we still do not know whether vitamin D deficiency is a direct consequence of the treatment (*i.e.*, higher catabolism of vitamin D) or it is due to the fact that this subgroup of patients had a more severe form of the disease. The scientific literature suggests a role for chemotherapy in the onset or worsening of hypovitaminosis D (47, 48). It is possible that this could be the consequence of an increase in BMI and adipose tissue observed in early-stage BC patients undergoing adjuvant chemotherapy (47). Additionally, it could be speculated that, during chemotherapy, patients feel unwell and therefore spend more time indoors, and finally, some treatments induce photosensitivity; hence, patients are advised to avoid sun exposure.

Our study has limitations. In clinical practice and in the present study, serum 25(OH) D concentrations are measured by CLIA, a method that can be affected by interference, potentially resulting in underestimations (49, 50), unlike liquid chromatography/mass spectrometry, which is currently considered the most accurate and precise method for measuring 25(OH)D (51, 52). Limitations of platform assays to measure serum 25(OH)D concentrations may impact guidelines and practice decision-making (53). According to the International Vitamin D Standardization Program, enzyme immunoassays with a coefficient of variation of ≤10% and an average of ≤5% are acceptable (54). The LIAISON® 25 OH Vitamin D TOTAL Assay utilized in our study is a Centers for Disease Control and Prevention-certified method (CDC Certified Vitamin D Assays-508) and approved by the "Vitamin D Protocol" certification program (52); furthermore, all vitamin D measurements were appropriately calibrated using calibrators integrated into the reagent cartridge.

A sun-exposure questionnaire tested on healthcare workers in southern Italy predicted circulating 25-(OH)D concentrations (55). Although patients with BC in Italy are advised to refrain from sun exposure during chemotherapy treatment due to drug-induced photosensitivity, we were able to account for sun exposure by taking into consideration the season of blood drawing; however, no substantial difference in results was observed after the seasonal adjustment of the original model. Furthermore, given the cross-sectional nature of our data, we cannot infer causality, and the associations we found could be biased by unknown factors or by the heterogeneity of the sample. The relevant limitations of this analysis of baseline data from the DEDiCa trial include exclusively descriptive analyses and unavoidable heterogeneity in terms of sun exposure, in addition to the dose and duration of vitamin D supplementation. However, even though the data were derived from a clinical trial, the number of subjects randomized is large enough to allow for a baseline cross-sectional analysis for explorative purposes, *i.e.*, to investigate which variables, if any, may cluster in the vitamin D sufficiency group and which in the insufficiency group.

CONCLUSIONS

In conclusion, this study shows that in a population of women residing in Italy and diagnosed with BC without metastasis within one year from diagnosis, the prevalence of vitamin D deficiency is still high, regardless of the subjects' age and the latitude of residence. Overweight or obese patients were the most at risk, as were those undergoing chemotherapy. Recent data on the potential protective effect of vitamin D on the risk of neoplastic recurrence and death and the frequent need for skeletal protection treatments, which are known to be less effective in hypovitaminosis D conditions (56), make it imperative for any physician treating these patients to ad-

equately address and treat vitamin D deficiency.

Contributions
All the authors made a substantial intellectual contribution, read and approved the final version of the manuscript, and agreed to be accountable for all aspects of the work.

Conflict of interest
AF, has received speaker's fees from Abiogen Pharma; LSAA, is a founding member of the International Carbohydrate Quality Consortium (ICQC), has received honoraria from the Nutrition Foundation of Italy (NFI) and research grants from Lega Italiana per la Lotta contro i Tumori (LILT), a non-profit organization for the fight against cancer; GA, has received speaker's fees from Eli Lilly, Galapagos, UCB and Amgen; DG, has received consulting fees from Accord Health Care, Abiogen, Amgen, Elililly, Neopharmed-Gentili, Organon and Novartis. No other authors declare conflicts of interest.

Ethics approval and consent to participate
The study was conducted following the Declaration of Helsinki; the protocol was approved by the Italian Ministry of Health, the Italian Medicine Agency (AIFA), and the ethics boards of each recruiting hospital (ClinicalTrials.gov NCT02786875).

Informed consent
Informed consent was obtained from all subjects involved in the study.

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Availability of data and materials
Data are available on the ZENODO public repository.

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