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Association of adiposity, dysmetabolisms, and inflammation with aggressive breast cancer subtypes: a cross-sectional study

Roberto Agresti[1] · Elisabetta Meneghini[2] · Paolo Baili[2] · Pamela Minicozzi[2] ·
Alberto Turco[2] · Ilaria Cavallo[2] · Francesco Funaro[2] · Hade Amash[2] ·
Franco Berrino[3] · Elda Tagliabue[4] · Milena Sant[2]

Abstract Obesity and metabolic syndrome are risk and prognostic factors for breast cancer (BC) and are associated with chronic inflammation. We investigated the association between distinct BC subtypes and markers of adiposity, dysmetabolisms, and inflammation. We analyzed 1779 patients with primary invasive BC treated at a single institution, for whom anthropometric and clinical-pathological data were archived. BC subtypes were classified by immunohistochemical staining of ER, PR, HER2, and Ki67, and their relations with the study markers were assessed by multinomial logistic regression. Adjusted odds ratios (ORs) and 95 % confidence intervals (CIs) were calculated taking luminal A as reference. All subtypes more aggressive than luminal A were significantly more frequent in younger (<45 years) than older women. Before menopause, luminal B HER2-negative tumors were positively associated with large waist (OR 2.55, 95 % CI 1.53–4.24) and insulin resistance (OR 1.90, 95 % CI 1.05–3.41); luminal B HER2-positive tumors with large waist (OR 2.11, 95 % CI 1.03–4.35) and triple-negative tumors with overweight (OR 3.04, 95 % CI 1.43–6.43) and high C-reactive protein (p trend = 0.026). In postmenopausal women aged <65, luminal B HER2-negative (OR 1.94, 95 % CI 1.16–3.24) and luminal B HER2-positive tumors (OR 2.48, 95 % CI 1.16–5.27) were positively related with metabolic syndrome. Dysmetabolisms and inflammation may be related to different BC subtypes. Before menopause, triple-negative cancers were related to obesity and chronic inflammation, and aggressive luminal subtypes to abdominal adiposity. After menopause, in women aged <65 these latter subtypes were related to metabolic syndrome. Control of adiposity and dysmetabolism can reduce the risk of aggressive BC subtypes, improving the prognosis.

Keywords Breast cancer subtype · Adiposity · Insulin resistance · Metabolic syndrome · Inflammation

Abbreviations
BC   Breast cancer
BMI   Body mass index
CI   Confidence interval
CRP   C-reactive protein
ER   Estrogen receptor
FISH   Fluorescence in situ hybridization
HER2   Human epidermal growth factor receptor 2
HOMA   Homeostatic model assessment index
IDC   Infiltrating ductal carcinoma
IHC   Immunohistochemical
ILC   Infiltrating lobular carcinoma
IQR   Interquartile range
IR   Insulin resistance
OR   Odds ratio
PR   Progesterone receptor

✉ Elisabetta Meneghini
elisabetta.meneghini@istitutotumori.mi.it

1   Breast Surgery Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milan, Italy

2   Analytic Epidemiology and Health Impact Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milan, Italy

3   Epidemiology and Prevention Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milan, Italy

4   Molecular Targeting Unit, Fondazione IRCCS Istituto Nazionale dei Tumori, Via Venezian 1, 20133 Milan, Italy

Introduction

Breast cancer (BC) is not one disease but several. Subtypes defined by distinct gene expression profiles differ in terms of natural history, response to treatment, and outcomes [1]. Subtypes defined by estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67 status approximate the intrinsic subtypes that have emerged from gene expression studies [2] and differ in their responses to treatment and outcomes [3, 4].

Women who are obese, have metabolic syndrome, hyperinsulinemia, or insulin resistance (IR) are at significantly greater risk of BC than women who do not have these disorders [5–8]. Survival is also worse for these women [9–11]. Some studies suggest that the influence of obesity and metabolic syndrome on BC risk varies with subtype and with menopausal status. Thus, in premenopausal women, high body mass index (BMI) has been consistently associated with a lower risk of hormone-positive BC [12–14], while in these same women abdominal rather than general adiposity has been associated with a higher risk of receptor-negative BC [14, 15]. In postmenopausal women, by contrast, high BMI has been associated with an increased risk of hormone-positive BC [12].

Abdominal obesity, hyperinsulinemia, IR, and metabolic syndrome are also associated with chronic inflammation [16]. However, only a few studies have investigated metabolic [17, 18] and inflammatory factors [19] in relation to the risk of specific BC subtypes.

The present cross-sectional study investigated whether markers of adiposity, dysmetabolism, and inflammation relate differently to distinct BC subtypes. We analyzed data from a prospectively maintained hospital-based registry of BC cases, stratifying patients by their menstrual status to take account of possible changes after menopause in adiposity, dysmetabolism, and inflammation.

Materials and methods

Study population

We analyzed data on BC cases prospectively archived in the BC registry of the National Cancer Institute (INT), Milan, Italy [20]. The registry, which started in October 2011, collects anthropometric, clinical, and pathological-biological data on all women with BC who present at the INT. Cases archived up to February 2015 with a diagnosis of primary invasive BC [21] who underwent surgery, not receiving any neoadjuvant treatment, were potentially eligible for inclusion in the study.

According to ICD-O-3 codes [22], tumor morphology was grouped in four categories: infiltrating ductal carcinoma (IDC) (8500), infiltrating lobular carcinoma (ILC) (8520), infiltrating mixed ductal and lobular (8522-8524), and Paget's disease (8540-8543). Tumors were classified as well differentiated, moderately differentiated, poorly differentiated, and unknown. BC subtypes were defined by ER, PR, HER2, and Ki67 status according to the 2011 St. Gallen Consensus Statement [2] as follows:

- Luminal A: ER- and/or PR-positive, HER2-negative, Ki67 <14 %;
- Luminal B (HER2-negative): ER- and/or PR-positive, HER2-negative, Ki67 ≥14 %;
- Luminal B (HER2-positive): ER- and/or PR-positive, HER2-positive, any Ki67;
- HER2-positive (non-luminal): ER- and PR-negative, HER2-positive, any Ki67;
- Triple-negative: ER- and PR-negative, HER2-negative, any Ki67.

Following American Association of Clinical Oncology guidelines [23], ER and PR were considered positive if immunostaining was seen on 1 % or more of tumor nuclei. HER2 was considered positive when (a) the immunohistochemical (IHC) score was 3+ or (b) the IHC was 2+ and fluorescence in situ hybridization (FISH) indicated gene amplification. HER2 was negative when the IHC score was 0–1+. HER2 was considered unknown for IHC scores 2+ and FISH unavailable. The tumor proliferation marker, Ki67 index, was evaluated by IHC visual assessment of Ki67-antigen expression in tissue sample fixed in 10 % buffered formalin. Specifically, the assessment was performed in a selected representative block of each tumor by selecting at least three fields of 'hot-spots' at the periphery of tumor edge of invasion and counting ratio between stained and unstained nuclei of about 500 cells.

The following markers of adiposity, dysmetabolism, and inflammation were analyzed:

- BMI, a measure of general adiposity [classified as <25 kg/m^2 (not overweight), 25 to <30 kg/m^2 (overweight), and ≥30 kg/m^2 (obese)];
- Waist circumference, a measure of abdominal adiposity (<80 and ≥80 cm);
- Fasting blood insulin and fasting blood glucose, divided into tertiles after excluding diabetic patients, who formed a separate category;
- Homeostatic model assessment index of insulin resistance (HOMA-IR);
- Metabolic syndrome;
- C-reactive protein (CRP; marker of inflammation).

HOMA-IR, calculated by multiplying fasting blood insulin (mU/L) by fasting blood glucose (mg/dL), and

dividing by 405 [24], was classified as <2.5 and ≥2.5. Metabolic syndrome was classified according to the definition proposed in the 2009 Joint Scientific Statement by International Diabetes Federation, National Heart, Lung and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity [25]. In accordance with the Joint Scientific Statement, we took any three of the following five conditions to indicate metabolic syndrome: waist circumference ≥80 cm; triglycerides ≥150 mg/dL or lipid-lowering medication for dyslipidemia; HDL cholesterol ≤50 mg/dL or medication for dyslipidemia; systolic blood pressure ≥130 mmHg or diastolic blood pressure ≥85 mmHg, or antihypertensive medication; fasting glucose ≥100 mg/dL [25]. CRP was classified as absent (0 mg/L) or present (≥1 mg/L), dividing present further as 1 mg/L (mild inflammation), 2–10 mg/L (moderate inflammation), and >10 mg/L (acute inflammation).

A total of 2054 cases were potentially eligible according to our initial selection criteria. Of these, 75 were excluded because of insufficient information to assign the BC subtype, 125 because of insufficient information to assess the presence of metabolic syndrome, 75 because BMI (7 cases) or information on blood insulin or lipids was missing (38 and 30 cases, respectively). Thus, 1779 cases were available for the analyses and divided into three categories according to their menstrual status. Women whose last menstruation was 12 months or more before diagnosis (810 cases, including 46 with iatrogenic menopause unrelated to BC) or who were 55 years old or more (290 cases) at diagnosis and information on last menstruation was lacking were considered postmenopausal. Women whose last menstruation was 3 months or less before diagnosis (565 cases) or who were under 45 years old (31 cases) and had no information on last menstruation were considered premenopausal. The remaining 83 women were aged 45–54 years; of these, 17 whose last menstruation was more than three but less than 12 months earlier were considered perimenopausal, and 66 with no information on last menstruation were assigned unknown menopausal status, although many were probably perimenopausal.

Statistical methods

Patient and disease characteristics by menstrual status were summarized by descriptive statistics. Differences in the distribution of characteristics between the premenopausal and postmenopausal groups were assessed by the Kruskal–Wallis test for continuous variables, and the χ^2 test or Fisher's exact test, as appropriate, for categorical variables, excluding women who were perimenopausal or whose menopausal status was unknown. Associations between BMI and other markers (waist circumference, CRP, hyperinsulinemia, IR, metabolic syndrome, and individual factors contributing to metabolic syndrome) were assessed by Spearman's rank correlation coefficient (ρ) or the χ^2 test, as appropriate. Associations of dysmetabolism markers and disease characteristics with BC subtypes were explored by multinomial logistic regression, making it possible to calculate the odds (as odds ratios, ORs) of events of interest simultaneously taking account of any other events. We used multinomial logistic regression to calculate the minimally adjusted odds of having one of the BC subtypes (luminal B HER2-negative, luminal B HER2-positive, HER2-positive non-luminal, or triple-negative) relative to the reference subtype (luminal A) for each disease characteristic and marker of dysmetabolism and inflammation, adjusting only for age and BMI (where appropriate). The analyses were done on pre- and postmenopausal cases separately, excluding cases with perimenopausal and unknown menopausal status. The Wald test was used to assess the significance of linear trends in ORs with increasing levels of categorical variables. The likelihood ratio test was used to compare models with and without the product term of the interacting variables. Two-sided p values <0.05 were considered significant.

Since there were significant interactions between IR and BMI for luminal B (HER2-negative) subtype, and between metabolic syndrome and age for luminal B (both HER2-negative and HER2-positive) subtypes for postmenopausal women, age-adjusted ORs for IR are presented in strata of BMI (<25 and ≥25 kg/m^2) and age- (continuous variable) and BMI-adjusted ORs for metabolic syndrome are presented in strata of age (<65 and ≥65 years).

To account for any reverse causation effect of disease stage on markers of inflammation [26], we did a stratified analysis to assess the association between CRP and BC subtype, doing multinomial logistic regression analyses separately on T1-T2N0 and more advanced cases. Fully adjusted models including age, BMI, metabolic syndrome, and CRP, within groups of tumor stage, were feasible only in the postmenopausal T1-T2N0 group, because of the small numbers of postmenopausal patients with more advanced stage and premenopausal patients with metabolic syndrome.

Analyses were done with the Stata statistical package, release 12.0 (Stata Corporation, College Station, TX, USA).

Results

Table 1 summarizes the characteristics of the 1779 BC cases, by menopausal status. The distributions of markers of adiposity, dysmetabolism, and inflammation differed

Table 1 General and tumor characteristics of 1779 breast cancer patients, by menopausal status

	Menstrual status						p^a
	Premenopausal		Postmenopausal		Perimenopausal/unknown		
	No.	%	No.	%	No.	%	
Total cases	596	33.5[b]	1100	61.8[b]	83	4.7[b]	
Median (IQR) age (years)[c]	45.2 (41.3–48.5)		66.1 (59.7–73.6)		51.7 (49.4–53.2)		–
BMI (kg/m^2)							
<25	465	78.0	512	46.6	52	62.7	<0.001[d]
25 to <30	100	16.8	392	35.6	23	27.7	
≥30	31	5.2	196	17.8	8	9.6	
Waist circumference (cm)							
<80	221	37.1	155	14.1	15	18.1	<0.001[d]
≥80	268	45.0	721	65.5	53	63.9	
Unknown	107	17.9	224	20.4	15	18.1	
Median (IQR) glucose (mg/dL)[f]	90 (85–97)		98 (90–106)		94 (88–99)		<0.001[e]
Median (IQR) insulin (μIU/mL)[f]	6.9 (4.9–9.7)		8.5 (6.1–11.9)		7.2 (5.6–10.2)		<0.001[e]
HOMA-IR index							<0.001[d]
<2.5	447	80.4	646	64.2	61	77.2	
≥2.5	116	19.6	360	35.8	18	22.8	
Diabetes							<0.001[g]
No	593	99.5	1006	91.5	79	95.2	
Yes	3	0.5	94	8.5	4	4.8	
Median (IQR) HDL cholesterol (mg/dL)[h]	67 (59–78)		65 (54–76)		67 (54–77)		<0.001[e]
Median (IQR) triglycerides (mg/dL)[h]	66 (52–84)		93 (71–121)		81 (59–115)		<0.001[e]
Lipid-lowering treatment							<0.001[g]
No	590	98.9	890	80.9	80	96.4	
Yes	6	1.1	210	19.1	3	3.6	
Blood pressure (mmHg)							
<130/85	501	84.1	352	32.0	60	72.3	<0.001[d]
≥130/85	68	11.4	244	22.2	9	10.8	
Hypertension treatment	27	4.5	504	45.8	14	16.9	
Metabolic syndrome							
No	555	93.1	563	51.2	65	78.3	<0.001[d]
Yes	41	6.9	537	48.8	18	21.7	
CRP (mg/L)							
0	255	42.8	176	16.0	23	27.7	<0.001[d]
1	203	34.1	369	33.5	36	43.4	
2–10	124	20.8	509	46.3	20	24.1	
>10	14	2.3	46	4.2	4	4.8	
BC subtype							
Luminal A	144	24.2	303	27.6	26	31.3	0.063[d]
Luminal B (HER2-negative)	300	50.3	583	53.0	36	43.4	
Luminal B (HER2-positive)	69	11.6	94	8.5	12	14.5	
Triple-negative	48	8.0	67	6.1	7	8.4	
HER2-positive (non-luminal)	35	5.9	53	4.8	2	2.4	
TN stage							
T1-T2N0	341	57.2	654	59.5	59	71.1	<0.001[d]
Other	233	39.1	351	31.9	21	25.3	
Unknown	22	3.7	95	8.6	3	3.6	

Table 1 continued

	Menstrual status						p^a
	Premenopausal		Postmenopausal		Perimenopausal/unknown		
	No.	%	No.	%	No.	%	
Grade							
Well differentiated	45	7.5	67	6.1	6	7.2	0.005[d]
Moderately differentiated	296	49.7	622	56.6	48	57.8	
Poorly differentiated	242	40.6	403	36.6	29	34.9	
Unknown	13	2.2	8	0.7	0	0.0	
Morphology							
Ductal	394	66.1	732	66.5	55	66.3	0.887[d]
Lobular	57	9.6	119	10.8	7	8.4	
Mixed	87	14.6	147	13.4	17	20.5	
Paget's disease	8	1.3	13	1.2	0	0.0	
Other invasive	50	8.4	89	8.1	4	4.8	

[a] p values excluding women with unknown or perimenopausal status

[b] Row percentages

[c] Median and interquartile range (IQR)

[d] Chi-square test

[e] Kruskal–Wallis test

[f] Median and IQR excluding diabetic cases

[g] Fisher's exact test

[h] Median and IQR excluding cases receiving medication for dyslipidemia

markedly between the 596 (33 %) premenopausal and 1100 (62 %) postmenopausal patients (all $p \leq 0.001$). Only 83 (5 %) cases were perimenopausal/unknown menopausal status. Compared to premenopausal cases, postmenopausal cases were more often overweight (36 vs. 17 %) or obese (18 vs. 5 %), more often had waist circumference $\geq$80 cm (66 vs. 45 %) and had higher median levels of fasting blood glucose (98 vs. 90 mg/dL), insulin (8.5 vs. 6.9 µIU/mL), and triglycerides (93 vs. 66 mg/dL). There were also more postmenopausal than premenopausal women with high blood pressure (22 vs. 11 %), antihypertensive treatment (46 vs. 5 %), lipid-lowering treatment (19 vs. 1 %), insulin resistance (HOMA-IR $\geq$2.5: 36 vs. 20 %), diabetes (9 vs. 1 %), metabolic syndrome (49 vs. 7 %), and moderate inflammation (CRP 2–10 mg/L: 46 vs. 21 %). Premenopausal women more often ($p < 0.001$) had advanced tumors (more than T1-T2N0, 39 vs. 32 %,) and less favorable ($p = 0.005$) grade distribution (50 vs. 57 % moderately differentiated; 41 vs. 37 % poorly differentiated) than postmenopausal cases. Differences between the two groups were not significant for BC subtype ($p = 0.063$) or BC morphology ($p = 0.887$).

BMI correlated strongly (Spearman's) with waist circumference in the postmenopausal ($\rho = 0.791$, $p < 0.001$) and premenopausal women ($\rho = 0.688$, $p < 0.001$). Overweight or obese women (BMI $\geq$25 kg/m^2) were more

likely to have CRP above zero (94 vs. 73 %, $p < 0.001$ in the postmenopausal group, and 87 vs. 49 %, $p < 0.001$ in the premenopausal group), and there were more cases of metabolic syndrome (63 vs. 32 %, $p < 0.001$ in the postmenopausal group, and 20 vs. 3 %, $p < 0.001$ in the premenopausal group) than among underweight or normal-weight women (data not shown).

The multinomial logistic regression results are shown in Table 2 for premenopausal women. All BC subtypes biologically more aggressive than luminal A were significantly more frequent among younger ($<$45 years) than older ($\geq$45 years) women. Luminal B (HER2-negative) disease was more frequent in women with large waist (OR 2.55, 95 % CI 1.53–4.24) than those with waist $<$80 cm; glucose in the second tertile of the distribution was more frequent (OR 2.04, 95 % CI 1.21–3.41), and the HOMA-IR index (indicating insulin resistance) was higher than in those with a low index (OR 1.90, 95 % CI 1.05–3.41). Luminal B HER2-positive disease was more frequent than luminal A in women with large waist (OR 2.11, 95 % CI 1.03–4.35) and women with high CRP (p trend = 0.048). Waist circumference was unknown in 18 % of women. We examined these cases as a separate category, and no association emerged.

Triple-negative disease was significantly more frequent than luminal A in overweight women (OR 3.04,

Table 2 Premenopausal women

	Total	Breast cancer subtype								
		Luminal A	Luminal B (HER2-negative)		Luminal B (HER2-positive)		Triple-negative		HER2-positive (non-luminal)	
		%	%	OR (95 % CI)	%	OR (95 % CI)	%	OR (95 % CI)	%	OR (95 % CI)
Age (years)										
<45	295	15.2	50.9	2.25 (1.47–3.42)	15.2	4.34 (2.35–8.01)	10.9	4.92 (2.42–9.98)	7.8	4.46 (2.03–9.82)
≥45	301	32.9	49.8	1	8.0	1	5.3	1	4.0	1
BMI (kg/m²)										
<25	465	25.4	51.2	1	11.2	1	6.7	1	5.6	1
≥25	131	19.8	47.3	1.30 (0.78–2.18)	13.0	1.78 (0.88–3.63)	13.0	3.04 (1.43–6.43)	6.9	1.89 (0.78–4.60)
Waist circumference (cm)										
<80	221	29.0	43.9	1	11.8	1	8.1	1	7.2	1
≥80	268	17.9	56.7	2.55 (1.53–4.24)	13.1	2.11 (1.03–4.35)	6.7	1.03 (0.42–2.53)	5.6	1.28 (0.50–3.27)
Unknown	107	29.9	47.7	1.10 (0.63–1.93)	7.5	0.61 (0.24–1.54)	11.2	1.03 (0.42–2.57)	3.7	0.46 (0.14–1.55)
CRP (mg/L)[a]										
0	255	28.2	51.0	1	10.6	1	5.1	1	5.1	1
1	203	22.2	50.3	1.38 (0.86–2.21)	10.8	1.51 (0.74–3.06)	9.9	2.62 (1.14–6.01)	6.9	1.93 (0.80–4.65)
2–10	124	20.2	50.0	1.59 (0.88–2.88)	13.7	2.27 (0.98–5.26)	9.7	2.82 (1.04–7.69)	6.4	2.08 (0.70–6.17)
p_{trend}				0.092		0.048		0.026		0.131
Glucose[b, c], tertiles										
I	204	27.9	44.1	1	14.2	1	7.8	1	5.9	1
II	198	18.2	52.5	2.04 (1.21–3.41)	11.1	1.41 (0.69–2.87)	10.6	2.37 (1.06–5.27)	7.6	2.32 (0.96–5.64)
III	191	26.2	54.4	1.48 (0.90–2.42)	9.4	0.81 (0.39–1.69)	5.8	0.81 (0.33–1.99)	4.2	0.87 (0.32–2.38)
p_{trend}				0.119		0.610		0.777		0.907
Insulin[c, d], tertiles										
I	201	27.4	49.2	1	10.4	1	7.0	1	6.0	1
II	195	26.2	47.7	1.06 (0.65–1.72)	11.8	1.25 (0.61–2.58)	7.2	1.08 (0.46–2.54)	7.2	1.29 (0.53–3.11)
III	197	18.8	53.8	1.59 (0.92–2.75)	12.7	1.60 (0.73–3.53)	10.2	1.52 (0.62–3.73)	4.6	0.88 (0.31–2.55)
p_{trend}				0.105		0.249		0.373		0.882
HOMA-IR index[c]										
<2.5	477	26.0	48.0	1	11.5	1	7.8	1	6.7	1
≥2.5	116	16.4	59.5	1.90 (1.05–3.41)	12.1	1.40 (0.62–3.16)	9.5	1.35 (0.55–3.29)	2.6	0.47 (0.12–1.76)
Metabolic syndrome										
No	555	25.1	50.1	1	11.4	1	7.6	1	6.0	
Yes	41	12.2	53.7	2.43 (0.87–6.75)	14.6	2.98 (0.82–10.83)	14.6	3.62 (0.97–13.56)	4.9	Not evaluable

Distribution (%) with age- and BMI-adjusted odds ratios (ORs) and 95 % confidence intervals (CI) of breast cancer subtypes in relation to markers of adiposity, dysmetabolism, and inflammation

ORs and 95 % CIs calculated by multinomial logistic regression modeling taking luminal A as reference

[a] Patients with acute inflammation (CRP >10 mg/L, 14 cases) were excluded from this analysis

[b] Tertiles excluding three cases with diabetes; tertile thresholds 87 and 94 mg/dL

[c] ORs excluding three cases with diabetes

[d] Tertiles excluding three cases with diabetes; tertile thresholds 5.5 and 8.5 μIU/mL

95 % CI 1.43–6.43), those with high CRP (ORs 2.62, 95 % CI 1.14–6.01 for low-grade inflammation; 2.82, 95 % CI 1.04–7.69 for moderate-grade inflammation; p trend = 0.026), and those in the second tertile of blood glucose (OR 2.37, 95 % CI 1.06–5.27). HER2-positive (non-luminal) disease was more frequent than luminal A in women in the second tertile of blood glucose (OR 2.32, 95 % CI 0.96–5.64).

All subtypes except HER2-positive (non-luminal), biologically more aggressive than luminal A, were non-significantly more frequent in women with metabolic syndrome.

Stratified analyses done separately on early and more advanced premenopausal BC confirmed the association between increasing CRP and triple-negative tumors only in the early-stage subgroup (T1-T2N0) (age- and BMI-adjusted ORs 3.73, 95 % CI 1.25–11.11 for low-grade inflammation and 4.70, 95 % CI 1.30–16.94 for moderate-grade inflammation; p trend = 0.012) (data not shown).

The multinomial logistic regression results for postmenopausal women are illustrated in Table 3. Luminal B (HER2-negative) disease was significantly more frequent than luminal A in overweight than not overweight women (OR 1.51, 95 % CI 1.14–2.00) and in women with diabetes (OR 1.94, 95 % CI 1.06–3.55) than among those in the lowest glucose tertile. Normal-weight women with IR had a lower frequency of luminal B (HER2-negative) than luminal A disease (OR 0.59, 95 % CI 0.35–1.01).

In younger postmenopausal women aged <65, luminal B cancers (HER2 negative and positive) were associated with metabolic syndrome significantly more frequently than luminal A (respectively OR 1.94, 95 % CI 1.16–3.24 and OR 2.48, 95 % CI 1.16–5.27). No significant associations were seen in older postmenopausal patients.

Triple-negative disease was significantly more frequent than luminal A in women in the second glucose tertile (OR 2.59, 95 % CI 1.31–5.14) than the first. No significant association was found between HER2-positive (non-luminal) disease and any indicator of dysmetabolism. Stratified analyses on postmenopausal CRP levels did not bring to light any association with tumor subtype in either early or more advanced stages.

The fully adjusted multinomial logistic regression model, run separately in younger and older postmenopausal patients with early-stage disease, confirmed the significant relations between metabolic syndrome and luminal B (both HER2-negative and -positive) found by the minimally adjusted model for younger women (luminal B HER2-negative OR 2.20, 95 % CI 1.14–4.27; luminal B HER2-positive OR 3.92, 95 % CI 1.57–9.80). BMI and the luminal B HER2-negative subtype showed a weaker relation, no longer significant (data not shown).

Discussion

This cross-sectional study examined how markers of dysmetabolism and inflammation were related with immunohistochemically defined disease subtypes in a consecutive series of 1779 BC patients diagnosed and treated at a single institute. Before menopause, BC subtypes more aggressive than luminal A were significantly more frequent among younger (<45 years) than older (≥45 years) women, while after menopause HER2-positive (non-luminal) disease was more frequent in the younger (<65 years) than older

(≥65 years) women. Similar findings have already been reported [27] and are in line with the generally poorer prognosis in younger than older BC patients [28]. Young age in premenopausal women was most strongly associated with the triple-negative, luminal B (HER2-positive), and HER2-positive (non-luminal) subtypes, which have considerably worse survival than luminal A disease, although this is now somewhat mitigated by the targeted treatments for HER2-positive disease.

We also found that more aggressive BC subtypes were associated with dysmetabolic markers, with some differences according to menstrual status. Previous studies reported that premenopausal obesity reduced the risk of ER- and PR-positive BC [12] and increased the risk of hormone-negative BC (which includes triple-negative) [15, 29, 30]. A recent study found that weight gain in premenopausal women raised the risk of ER-positive and PR-negative and ER- and PR-negative rather than ER- and PR-positive BC [31]. In agreement with these etiological studies, we found that overweight/obesity was significantly associated with the triple-negative subtype in premenopause.

In addition, large waist circumference (adjusted by BMI in the multinomial regression model) was significantly associated with luminal B (HER2-negative and positive) premenopausal BC. Hip circumference and waist-to-hip ratio (indicators of central adiposity) are reported as BC risk factors before menopause [32, 33], suggesting a role of IR. This resistance slows the intake of blood glucose into the cells, with a consequent rise in glycemia, which in turn stimulates insulin secretion (by feed-back regulation). Insulin is a mutagenic agent and hyperinsulinemia lowers the levels of insulin-like growth factor (IGF) 1- and 2-binding protein, increasing the bioavailability of IGF which is known to promote human breast cancer [34]. Insulin may also promote breast cancer growth by inducing aromatase activity and reducing sex hormone-binding globulin production with a consequent increase of mutagenic free estradiol [8].

In postmenopausal women, obesity was associated with luminal B (HER2-negative) BC and in younger postmenopausal women (<65 years) metabolic syndrome was significantly associated with luminal disease (HER2-negative and positive). Obesity is a documented risk factor for postmenopausal hormone receptor-positive BC [12]. The current interpretation of the causal mechanism is that after menopause adipose tissue is the main site of aromatase activity and the obesity-induced reduction in the production of sex hormone-binding globulin may further raise levels of circulating free estrogens, inducing ER and PR expression and stimulation of hormone receptor-positive tumors [35]. This causal relation between obesity and the risk of hormone receptor-positive subtypes might explain

Table 3 Postmenopausal women

	Total	Breast cancer subtype								
		Luminal A	Luminal B (HER2-negative)		Luminal B (HER2-positive)		Triple-negative		HER2-positive (non-luminal)	
		%	%	OR (95 % CI)	%	OR (95 % CI)	%	OR (95 % CI)	%	OR (95 % CI)
Age (years)										
<65	495	26.5	50.5	1.02 (0.77–1.35)	9.9	1.45 (0.91–2.31)	6.9	1.36 (0.80–2.32)	6.3	1.91 (1.05–3.45)
≥65	605	28.4	55.0	1	7.4	1	5.5	1	3.6	1
BMI (kg/m^2)										
<25	512	31.3	48.4	1	9.0	1	6.6	1	4.7	1
≥25	588	24.3	57.0	1.51 (1.14–2.00)	8.2	1.20 (0.76–1.92)	5.6	1.11 (0.65–1.90)	4.9	1.43 (0.79–2.57)
Waist circumference (cm)										
<80	155	32.3	45.2	1	11.0	1	7.7	1	3.9	1
≥80	721	26.9	54.2	1.17 (0.75–1.81)	8.2	0.82 (0.41–1.63)	6.0	0.89 (0.41–1.95)	4.7	1.36 (0.50–3.69)
Unknown	224	26.3	54.5	1.25 (0.76–2.06)	8.0	0.85 (0.38–1.88)	5.4	0.83 (0.33–2.09)	5.8	1.77 (0.60–5.22)
CRP (mg/L)[a]										
0	176	27.8	48.9	1	9.7	1	8.5	1	5.1	1
1	369	32.8	49.1	0.79 (0.52–1.21)	8.9	0.77 (0.39–1.54)	4.3	0.44 (0.20–0.98)	4.9	0.79 (0.33–1.91)
2–10	509	24.0	56.8	1.17 (0.76–1.81)	8.4	0.99 (0.49–1.99)	6.3	0.90 (0.42–1.93)	4.5	0.98 (0.39–2.42)
p_{trend}				0.183		0.829		0.759		0.901
Glucose[b], tertiles										
I	344	31.1	50.6	1	9.3	1	4.4	1	4.6	1
II	341	24.6	52.2	1.27 (0.89–1.81)	8.8	1.21 (0.68–2.15)	8.8	2.59 (1.31–5.14)	5.6	1.54 (0.75–3.20)
III	321	29.6	53.3	1.01 (0.70–1.44)	7.5	0.85 (0.46–1.58)	5.6	1.39 (0.65–2.97)	4.0	0.92 (0.41–2.07)
p_{trend}				0.886		0.637		0.366		0.891
Diabetes	94	18.1	63.8	1.94 (1.06–3.55)	8.5	1.66 (0.64–4.32)	4.3	1.81 (0.52–6.25)	5.3	2.15 (0.67–6.90)
Insulin[c,d], tertiles										
I	337	31.4	48.7	1	9.5	1	6.8	1	3.6	
II	339	27.7	53.4	1.16 (0.82–1.66)	6.8	0.80 (0.43–1.48)	6.2	1.03 (0.53–2.00)	5.9	1.79 (0.82–3.90)
III	330	26.1	53.9	1.13 (0.77–1.67)	9.4	1.16 (0.62–2.18)	5.8	1.02 (0.49–2.13)	4.8	1.44 (0.60–3.44)
p_{trend}				0.518		0.677		0.955		0.420
HOMA-IR index[d]										
Not overweight										
<2.5	403	30.0	49.9	1	9.4	1	6.5	1	4.2	1
≥2.5	85	38.8	38.8	0.59 (0.35–1.01)	7.1	0.57 (0.22–1.47)	9.4	1.15 (0.47–2.78)	5.9	1.13 (0.39–3.32)
Overweight										
<2.5	243	28.0	53.9	1	7.0	1	6.2	1	4.9	1
≥2.5	275	23.3	57.4	1.27 (0.84–1.92)	9.1	1.53 (0.75–3.11)	5.1	0.99 (0.44–2.20)	5.1	1.21 (0.52–2.82)
$p_{interaction}$				0.043		0.085		0.570		0.875
Metabolic syndrome										
≥65 years										
No	310	31.9	45.8	1	8.1	1	7.1	1	7.1	1
Yes	185	17.3	58.4	1.94 (1.16–3.24)	13.0	2.48 (1.16–5.27)	6.5	1.75 (0.72–4.29)	4.9	1.16 (0.45–3.01)
≥65 years										
No	253	28.1	51.8	1	10.3	1	5.9	1	4.0	1
Yes	352	28.7	57.4	0.99 (0.67–1.47)	5.4	0.57 (0.29–1.14)	5.1	0.78 (0.36–1.69)	3.4	0.75 (0.30–1.90)
$p_{interaction}$				0.038		0.001		0.260		0.744

Distribution (%) with age- and BMI-adjusted odds ratios (ORs) and 95 % confidence intervals (CIs) of breast cancer subtype in relation to markers of adiposity, dysmetabolism, and inflammation

ORs and 95 % CIs were calculated by multinomial logistic regression modeling, taking luminal A as reference

[a] Patients with acute inflammation (CRP >10 mg/L, 46 cases) were excluded from this analysis

[b] Tertiles excluding 94 cases with diabetes; tertile thresholds 93 and 103 mg/dL

[c] Tertiles excluding 94 cases with diabetes; tertile thresholds 6.9 and 10.4 μIU/mL

[d] ORs excluding women with diabetes

why after menopause we find no clear association between obesity and BC subtypes more aggressive than the luminal A.

Metabolic syndrome is a documented risk factor for BC, mainly in postmenopausal women [6, 7]. However, only few studies have analyzed metabolic syndrome in relation to specific BC subtypes. In our study, metabolic syndrome was associated with luminal B HER2-negative and luminal B HER2-positive disease in postmenopausal women under 65 years.

One important finding of the present study is the significant association between high CRP (a marker of chronic inflammatory status which is frequently high in persons with IR) and triple-negative and luminal B HER2-positive BC in premenopause. The standard CRP cut-off of 3 mg/L (separating low from high risk of cardiovascular disease) was not used because only a few cases were ≥ 3 and ≤ 10 mg/L, mainly premenopausal women—10 % (postmenopausal women 30 %). Systemic inflammation biomarkers such as CRP have been associated both with cancer risk [36] and tumor progression [37]. A chronic inflammatory status such as that related to obesity, hyperglycemia, or IR could cause oxidative damage and inactivate proteins involved in DNA repair or apoptotic control, thus promoting cancer cell initiation and growth [38]. It is also possible that the host's immune response is a consequence of the tumor growth itself [26, 39], and there may be a reverse causation effect. Stratified analyses done separately in early and more advanced stages confirmed the association between increasing CRP and triple-negative tumors in premenopausal BC only in the early-stage subgroup (T1-T2N0); this suggests that high CRP levels may be a consequence of the tumor growth, and limits the possibility of identifying potential relations between chronic inflammation and tumor subtypes in patients with advanced disease.

The association between dysmetabolic factors and more aggressive BC subtypes than the referent category of luminal A is in line with the reported detrimental effect on BC prognosis and survival of obesity [9], high insulin levels [11], CRP [40], and metabolic syndrome [10]. Our findings also agree with a recent review on BC heterogeneity and known risk factors, reporting that in premenopause obesity is negatively associated with luminal A subtypes and positively associated with triple-negative disease, whereas in postmenopausal women there are no clear relations between triple-negative subtype risk and obesity [30]. However, that review dealt with established BC risk factors such as reproductive factors, obesity, exogenous hormones, alcohol use, and family history, mainly affecting the risk of luminal A subtype, and the authors highlighted the need for subtype-specific studies on non-hormonal risk factors, including lifestyle factors and exogenous exposures, to clarify the etiology and the mechanism of action of less common subtypes, especially ER-negative ones [30].

In summary, in premenopausal patients overweight and chronic inflammation raised the odds of being diagnosed with triple-negative tumor; IR was associated with higher odds of luminal B HER2-negative subtype. After menopause, metabolic syndrome was associated with higher odds of luminal B HER2-negative and positive subtypes, for women younger than 65. These findings suggest that dysmetabolisms may play a role in the biological determinism of BC subtypes and further investigation is needed on the etiological mechanisms, as well as the roles of inflammatory biomarkers in tumor etiology and progression.

Measures against obesity in young women are important, given the increasing prevalence of overweight among young Italians [41]. Control of metabolic syndrome can help limit the incidence of the very frequent hormone-positive BC subtypes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study has been approved by the Ethical Committee of the National Cancer Institute.

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