Obesity and response to neoadjuvant chemotherapy in breast cancer: implication of the apelinergic system.

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Abstract

Obese subjects present higher risk of developing mammary tumors, worse disease free survival and altered response to neoadjuvant chemotherapy (NAC). The circulating levels of the apelin adipokines are increased in obese subjects and are associated with poorer prognosis in cancer patients. In this study, we showed that obesity and tumoral apelin expression are two factors associated with incomplete response to NAC in breast cancer patients.

Keywords:
Obesity-cancer link, apelin, neoadjuvant chemotherapy
**Introduction**

Breast cancer (BC) is the most common cancer and the leading cause of cancer death among women (1). It is now recognized that obesity, a condition that has reached pandemic proportions, is a risk factor for BC (2). Several potential mechanisms linking obesity and cancer have been identified, including the altered adipokines secretion (3, 4). Obese patients have increased circulating levels of the adipokine apelin (5, 6). We recently demonstrated in an *in vivo* study that this adipokine is implicated in the relation between obesity and BC (7). Reproducing obesity-related levels of apelin is sufficient to promote BC growth and metastatization (7). Besides promoting BC, recent data showed that obesity affects response to neoadjuvant or adjuvant chemotherapy for BC patients (8). As tumor apelin expression or its receptor APJ have been associated with poor survival in humans (9), we hypothesized that the apelinergic system could also be implicated in the adverse relation between obesity and pathological complete response (pCR) to NAC in BC patients.

**Patients and methods**

**Patients**

We retrospectively collected a series of 62 patients with early BC, treated with NAC at Cliniques universitaires Saint-Luc (a tertiary care center in Brussels, Belgium) between 2012 and 2020. Patients had received the same chemotherapy regimen and had remaining pre-treatment tumor samples available. Baseline information at diagnosis included anthropometric measurements, menopausal and diabetic status.

**Tumors biopsies**

Patients underwent biopsy at diagnosis. Hormone receptor status (estrogen receptor alpha and progesterone receptor) was evaluated by immunohistochemistry (IHC) and reported with the Allred score. Human epidermal growth factor receptor 2 (HER2) gene amplification status was determined by IHC and considered as positive for a staining superior to 10%. Subtypes are categorized as following: hormone receptor positive (HR+), HR+ HER2+, HER2+ and triple negative. Histological grade was assessed by the Nottingham scoring system. High Ki-67 index was determined for IHC staining above 15%. Nodes involvement at diagnosis was assessed by clinical evaluation or by cytopunction.

**Neo-adjuvant regimen**

Patients underwent neo-adjuvant regimen including combination of anthracycline and taxanes. Depending on the recommendations at the time of diagnosis, patients have received either: 4 cycles
of 5-fluorouracile (500mg/m²), epirubicin (100mg/m²), cyclophosphamide (500mg/m²) followed by 4 cycles of docetaxel (100mg/m²) or 4 cycles of epirubicin (90mg/m²) and cyclophosphamide (600mg/m²) followed by 12 cycles of paclitaxel (80mg/m²). Trastuzumab (6mg/m²) was administered if case of HER2 positive status.

**Immunohistochemistry**

Biopsies were fixed in 4% paraformaldehyde for 24h at room temperature before processing for paraffin embedding. Sections of 5µm were submitted to endogenous peroxidases inhibition. Sections were then subjected to antigen retrieval in 10 mM citrate buffer pH 5.7 and to blocking of aspecific antigen binding sites (TBS containing 5% BSA and 0.05% Triton). Anti-apelin and anti-APJ primary antibody (Apelin: Abcam59469, APJ: Abcam214369) were incubated in TBS containing 1% BSA and 0.05% Triton and detected with anti-rabbit horseradish peroxidase-conjugated polymer secondary antibodies (Agilent) overnight at 4°C. HRP was then visualized by DAB (Agilent). Cell nuclei were counterstained with hematoxylin. Stained slides were then digitalized using a SCN400 slide scanner (Leica Biosystems) at 40× magnification and tumor area were detected by a Pathologist. Percentage of stained tissue was analyzed using Visiopharm software.

**Statistical methods**

Statistical analyses were performed using Graphpad Prism 8.0. For descriptive analyses, categorical parameters were presented as distribution of frequencies and continuous parameters as mean +/- standard deviation. Descriptive analyses were performed using Chi-square test for categorical parameters and one-way ANOVA for continuous parameters. Factor associations with pathological complete response were tested by univariate and multivariate logistic regression. A p-value of ≤ .05 was considered significant.

**RESULTS**

**Patients’ characteristics**

Body mass index (BMI) at diagnosis was used to classify the 62 patients into three categories: normal weight (BMI < 25 kg/m²), overweight (BMI 25-30 kg/m²) and obese (BMI > 30 kg/m²) (Table 1). Thirty-five percent of patients were of normal weight, 40% were overweight and 25% were obese. The average age of patients at diagnosis was 52.6 ± 12.4 years, (range 33 to 75 years old). There was a trend towards overrepresentation of older patients in the overweight group (56.8 years ± 9.96) as compared to the normal weight group (49.1 years ± 13.73). This tendency was not observed in the obese category (50.5 years ± 12.86). Altogether, age did not correlate with BMI in this cohort (Figure 1). The overweight group was enriched in post-menopausal women, compared to normal weight and obese patients (72%
- 41% - 40% respectively, p=0.01). Two obese patients (3%) were diabetic. The majority of BC cases in the normal weight and obese groups were luminal. BC subtype was not significantly different between the subgroups. However, the overweight BC group was numerically enriched in triple negative BC cases, compared to normal weight and obese patients (36% - 18% - 7% respectively). Tumor size and cell proliferation were not significantly different among the three BMI categories. The majority of BC were of grade III. Nevertheless, tumor grade significantly diverged between the three subgroups, with a lower proportion of high-grade tumors in the obese patients (60%). Node infiltration did not differ between the subgroups.

The pCR rate was significantly different between the three subgroups, with a trend towards decreased efficacy of chemotherapy with increasing BMI category.

**Insertion TABLE 1**

![Age - BMI](image)

**Figure 1**: Correlation between age and BMI of patients (N=62).

**BMI and tumor apelin are independently associated with NAC pCR in BC**

We investigated the link between pCR after NAC and BMI by univariate and multivariate logistic regression, accounting for several parameters known to potentially affect pCR. These parameters were BMI, menopausal status, tumor grade, tumor size, nodal involvement and hormone receptor expression (Table 2). Moreover, we analyzed tumoral expression of the adipokine apelin and its receptor APJ, as our team recently highlighted in mouse models that obesity promotes tumor apelin expression and that high circulating apelin favors BC aggressiveness(7). Interestingly, only BMI (Odds ratio (OR) of 0.86, 95% confidence interval (CI) 0.74-0.99) and tumor apelin expression (OR of 0.90, 95% CI 0.83-0.97) were significantly associated with pCR in the multivariate analysis. No other factor
was significantly associated with pCR. Tumor apelin did not correlate with BMI (Figure 2), suggesting these two parameters might affect pCR independently.

|                    | Univariate analysis | Multivariate analysis |
|--------------------|---------------------|-----------------------|
|                    | Odds ratio (95% CI) | p-value               | Odds ratio (95% CI) | p-value               |
| BMI                | 0.88 (0.78 - 0.99)  | 0.03                  | 0.86 (0.74 – 0.99)  | 0.04                  |
| Postmenopausal     | 1.21 (0.44 - 3.39)  | 0.72                  | 0.85 (0.23 – 2.98)  | 0.80                  |
| High grade (III)   | 2.72 (0.73 - 13.2)  | 0.14                  | 2.49 (0.47 – 15.67) | 0.30                  |
| Size > 2cm         | 1.71 (0.56 – 5.67)  | 0.35                  | 1.52 (0.36 – 6.73)  | 0.57                  |
| Node               | 1.02 (0.35 – 3.10)  | 0.97                  | 1.73 (0.42 – 7.76)  | 0.46                  |
| HR+                | 0.59 (0.21 – 1.66)  | 0.31                  | 0.51 (0.13 – 1.90)  | 0.32                  |
| Apelin tumoral     | 0.95 (0.88 – 1.00)  | 0.06                  | 0.90 (0.83 – 0.97)  | 0.01                  |
| APIJ tumoral       | 1.02 (0.96 – 1.07)  | 0.59                  | 1.04 (0.98 – 1.13)  | 0.22                  |

**Table 2:** Univariate and multivariate logistic regression of clinical factors and odds ratio of pathological complete response (N=62). BMI: Body mass index, HR+: hormone receptor positive, APIJ: apelin receptor.

![Apelin - BMI](image)

**Figure 2**: Correlation between tumor apelin expression and BMI of patients (N=62).

**Discussion**

In this study, we retrospectively assessed the efficacy of anthracycline and taxanes-based NAC in 62 early BC patients. We explored whether weight status was associated with pCR rate. BMI was significantly associated with a poorer response to NAC in this cohort. We also observed numerical but not statistically significant trends towards higher pCR rate in high-grade tumors, and lower pCR rate in luminal tumors.
To further explore this association, we measured the tumoral expression of apelin and APJ. Interestingly, we discovered that high tumor apelin expression was significantly associated with a lower rate of pCR in these patients. This finding is a novel factor adding to the growing list of evidence that this adipokine has a detrimental role in cancer. Indeed, several preclinical and clinical studies have shown that apelin correlates with metastatization and poor overall survival (9, 10). Here, our clinical exploratory study suggests that tumor expression of apelin in BC is also associated with a poor response to NAC, a factor associated with worse disease-free survival (11).

In our previous preclinical study, we found that BC tumors developing in obese mice display an increased tumoral apelin expression. In the current cohort, BMI and tumor apelin did not correlate, suggesting these two parameters might affect pCR independently. This should be interpreted cautiously, as only 3% of patients were diabetic in our cohort, whereas the mice we used in preclinical studies were diabetic. Indeed, insulin is the main inducer of apelin expression (12) and could explain why obese subjects have increased apelin expression levels.

The small sample size and retrospective nature of our study are other limitations. These findings are thus hypothesis generating and must be validated in an independent, prospective cohort. In addition, a new study would allow us to refine our model by including other obesity-related parameters such as insulin sensitivity and circulating levels of apelin expression. Indeed, in a retrospective study, circulating levels of apelin were directly correlated with cancer stage in several different forms of tumors, including BC (13). Moreover, even if the use of BMI as a parameter for obesity is used in daily clinical practice, the use of waist-to-hip ratio as parameter for central obesity could be more appropriate to study the implication of obesity and apelin in response to NAC in BC patients.

In conclusion, in this retrospective exploratory study on 62 early BC patients treated with taxane and anthracycline-based NAC, BMI and tumor apelin expression were significantly and independently associated with poorer pCR rates. This observation supports the notion that besides their role in development of BC, both obesity and specific adipokines could play a role in the response to chemotherapy.
**Declarations**

**Ethics approval and consent to participate**

This study was approved by the Ethics Committee of Cliniques universitaires Saint-Luc (2017/25JUL/376).

**Fundings**

BJ is research director and PDC is a senior research associate at FRS-FNRS (Fonds de la Recherche Scientifique), Belgium. This work was supported by the Fonds de la Recherche Scientifique (FNRS FRFS-WELBIO) under the grants WELBIO-CR-2019C-02R. PDC is a recipient of the Funds Baillet Latour (Grant for Medical Research 2015). F.P. Duhoux received a postdoctoral clinical mandate (2017-034) from the not-for-profit organization ‘Foundation Against Cancer’ (Brussels, Belgium). Florian Gourgue is a FRIA grant holder of the FRS-FNSR, Belgium. F. Derouane received a doctoral mandate from the Breast Clinic, King Albert II Cancer Institute, Cliniques universitaires Saint-Luc. CVM received a post-doctoral clinician-researcher FRC mandate from the Cliniques universitaires Saint-Luc.

**Authors’ contributions**

FG, FD, FPD, PDC, BFJ conceived and designed the study. FG, FD, CVM, EV, HD, CB developed the methodology. FG, FD, CVM, LD analyzed and interpreted the data. FG, FD, CVM, FDP, PDC, BFJ wrote, reviewed, and/or revised the manuscript. All authors have read and approved the manuscript.

**Acknowledgments**

We thank Michele de Beukelaer from the 2IP imaging platform of UCLouvain for technical assistance.

**Abbreviation**

| Abbreviation | Description                  |
|--------------|------------------------------|
| APJ          | Apelin receptor              |
| BC           | Breast Cancer                |
| BMI          | Body mass index              |
| HER2         | Human epidermal growth factor receptor 2 |
| HR           | Hormone receptor             |
| IHC          | Immunohistochemistry         |
| NAC          | Neoadjuvant chemotherapy     |
| pCR          | Pathological complete response |

**Consent for publication**

Not applicable.
Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

The data that support the findings of this study are available from the corresponding authors upon request.

Table 1: Clinical characteristics of patients based on BMI category (N=62)

|                     | Normal Weight (BMI 18.5-24.9) | Overweight (BMI 25-29.9) | Obese (BMI ≥ 30) | Total (N=62) | p-value |
|---------------------|--------------------------------|--------------------------|------------------|--------------|---------|
| **BMI repartition** | 22 (35%)                       | 25 (42%)                 | 15 (22%)         | 02 (100%)    |         |
| **Mean age (year) + SD** | 46.1 ±13.74                   | 56.8 ±9.96               | 50.5 ±12.86      | 52.6 ±12.43  | 0.16    |
| **Menopausal status** | Premenopausal 13 (59%)         | 7 (28%)                  | 9 (60%)          | 29 (47%)     | 0.01    |
|                      | Postmenopausal 9 (41%)          | 18 (72%)                 | 6 (40%)          | 33 (53%)     |         |
| **Type II diabetic** | Yes 0 (0%)                      | 0 (0%)                   | 2 (13%)          | 2 (3%)       | *       |
|                     | No 22 (100%)                    | 25 (100%)                | 13 (87%)         | 60 (97%)     |         |
| **Molecular subtype** | HR+ 9 (41%)                     | 7 (28%)                  | 10 (60%)         | 26 (42%)     | 0.18    |
|                     | HR+ HER2+ 5 (23%)               | 5 (23%)                  | 1 (7%)           | 11 (18%)     |         |
|                     | HER2+ 4 (18%)                   | 4 (19%)                  | 3 (20%)          | 11 (18%)     |         |
|                     | TNBC 4 (18%)                    | 9 (39%)                  | 1 (7%)           | 14 (22%)     |         |
| **High Tumor size ≥2cm** | Yes 17 (77%)                   | 18 (72%)                 | 8 (53%)          | 43 (69%)     | 0.28    |
|                     | No 5 (23%)                      | 7 (28%)                  | 7 (47%)          | 19 (31%)     |         |
| **Tumor grade** | I-II 1 (5%)                     | 6 (24%)                  | 6 (40%)          | 13 (21%)     | 0.03    |
|                     | III 21 (65%)                    | 19 (76%)                 | 9 (60%)          | 49 (79%)     |         |
| **High Ki67 ≥15%** | Yes 26 (81%)                    | 13 (57%)                 | 15 (100%)        | 58 (94%)     | 0.49    |
|                     | No 2 (6%)                       | 2 (8%)                   | 0 (0%)           | 4 (6%)       |         |
| **Nodal infiltration** | Yes 14 (64%)                    | 16 (64%)                 | 12 (80%)         | 42 (68%)     | 0.51    |
|                     | No 8 (36%)                      | 9 (36%)                  | 3 (20%)          | 20 (32%)     |         |
| **pCR** | Yes 12 (55%)                     | 11 (44%)                 | 2 (13%)          | 25 (40%)     | 0.03    |
|                     | No 10 (45%)                      | 14 (56%)                 | 13 (87%)         | 33 (50%)     |         |

BMI: Body-mass index, SD: standard deviation, pCR: pathological complete response, HR: hormone receptor, TNBC: triple negative breast cancer. Anova one-way analysis for mean age, Chi square test for other parameters. P-values are annotated.

* Too few parameters to perform a Chi-square analysis.
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