Published OnlineFirst January 8, 2013; DOI: 10.1158/1055-9965.EPI-12-1020

Cancer Epidemiology, Biomarkers & Prevention

Research Article

Serum Amyloid A Is Associated with Obesity and Estrogen Receptor–Negative Tumors in Postmenopausal Women with Breast Cancer

Aline Barros Santana[1], Maria Salete Costa Gurgel[2,3], Joelma Ferreira de Oliveira Montanari[3], Flavia Muraro Bonini[1], and Silvia de Barros-Mazon[1]

Abstract

Serum amyloid A (SAA) is an acute-phase protein and also an adipokine, which has been associated with the development and prognosis of breast cancer. In the present study, we investigated the association between obesity and SAA in postmenopausal women with breast cancer and its relationship with clinicopathologic characteristics of tumors. Patients were grouped as nonobese or overweight/obese based on body mass index (BMI) plus waist circumference measurement. Serum SAA concentrations were determined by high-sensitivity micro-latex agglutination tests, detected by nephelometry. Serum SAA concentrations were higher in overweight/obese ($P = 0.008$) patients and this condition was dependent on obesity (BMI and waist circumference), as further shown by multivariate linear regression analysis done for SAA ($P = 0.01$). Concentrations of SAA were also higher in patients with estrogen receptor–negative (ER^-) tumors than in those with estrogen receptor–positive (ER^+; $P = 0.033$). Our results suggest a possible role for SAA in the development and prognosis of obesity-related breast cancer. A follow-up study of this population to assess overall and disease-free survival is in course and should bring contribution to evaluate the clinical role of SAA in breast cancer in the context of obesity. *Cancer Epidemiol Biomarkers Prev; 22(2); 270–4. ©2013 AACR.*

Introduction

Breast cancer is the second most frequent type of cancer worldwide and the most common among women (1). Alongside this, obesity is a known independent risk factor for breast cancer development (2) and has been associated with poor prognosis (3).

Because adipose tissue is the major tissue site of aromatose conversion of androstenedione to estrogen in postmenopausal women (4) and as estrogen stimulates cell proliferation, one ascribed mechanism to explain the influence of obesity on postmenopausal breast cancer development is the estrogen pathway (5).

However, the current recognition of the adipose tissue as an endocrine organ (3, 6) and the chronic low-grade inflammation associated with obesity in this tissue (7, 8) brings to light other mechanisms to explain the influence of obesity on postmenopausal breast cancer development. White adipose tissue produces several bioactive molecules, such as adipokines, inflammatory cytokines [TNF-α, interleukin-1 (IL-1), IL-6], and acute-phase protein serum amyloid A (SAA; refs. 7, 8).

All those inflammatory molecules are elevated in obesity and have also been associated with the onset and progression of breast cancer, including SAA (9–11). SAA is a nonspecific acute-phase protein with primary production ascribed to hepatocytes, following inflammatory stimuli by cytokines, including IL-1, IL-6, and TNF-α (12, 13). More recently it has been shown that it is also secreted by adipose tissue following cytokine stimulation (14, 15).

Because previous reports have shown that patients with breast cancer presented higher serum levels of SAA than controls with highest levels in patients bearing more advanced tumors (10) and that elevated concentrations of SAA were significantly associated with reduced overall survival (16), it has been suggested that SAA could be a prognostic factor useful for risk assessment and follow-up of the disease progression and overall survival.

Therefore, given the inflammatory state in obesity and its relationship with breast cancer, the aim of this study was to investigate whether obesity can influence serum SAA concentration in postmenopausal women bearing untreated breast cancer and the relationship of this protein with tumor characteristics.

Authors' Affiliations: Departments of [1]Clinical Pathology and [2]Obstetrics and Gynecology, Faculty of Medical Sciences; and [3]Center for Integral Attention to Women's Health (CAISM), State University of Campinas (Unicamp), Campinas, Sao Paulo, Brazil

Corresponding Author: Silvia de Barros-Mazon, State University of Campinas, R. Tessalia Vieira de Camargo, 126, Campinas, SP 13083-887, Brazil. Phone: 55-19-3521-9435; Fax: 55-19-3521-9434; E-mail: sbmazon@fcm.unicamp.br

doi: 10.1158/1055-9965.EPI-12-1020

Published OnlineFirst January 8, 2013; DOI: 10.1158/1055-9965.EPI-12-1020

Material and Methods

Study population, blood, and data collection

This study was conducted at the Center for Integral Attention to Women's Health (Caism)—State University of Campinas (Unicamp; Campinas, SP, Brazil). From November 2009 to September 2011, upon informed consent, 73 postmenopausal women (ages 45–80 years), who were undergoing surgery for primary invasive breast cancer were assessed for anthropometric measures. The groups were classified according to body mass index (BMI) plus abdominal fat absence or presence, based on waist circumference (WC) measurement. The nonobese group was composed of normal BMI (18.5–24.9 kg/m^2) plus normal waist circumference ($\leq$88 cm) patients ($n = 19$), and the overweight/obese group was composed of overweight (BMI $\geq$ 25 kg/m^2) plus obese (BMI $\geq$ 30 kg/m^2) patients with abdominal fat (waist circumference $>$ 88 cm; $n = 54$). Patients were considered postmenopausal when they had experienced absence of menstruation for 12 consecutive months. BMI (kg/m^2) was estimated by the ratio between weight (kg) and height (m^2) and categorized according to the classification of the World Health Organization (WHO; ref. 17): normal weight—BMI, 18.5 to 24.99 kg/m^2; overweight—BMI, 25.0 to 29.9 kg/m^2; and obesity—BMI, $\geq$30 kg/m^2. The waist circumference measurement was made at the height of the umbilicus; the cutoff point for waist circumference and the waist/hip ratio (WHR) were obtained according to the WHO recommendations. The clinicopathologic information of patients, that is, tumor–node–metastasis (TNM) staging, estrogen receptor (ER) and progesterone receptor (PR) status, and HER2 expression were collected by medical chart review. All patients had their blood collected at fasting for SAA quantification. Serum aliquots were stored at −80°C until use. The exclusion criteria included: BMI less than 18.5, previous chemotherapy and/or radiotherapy, presence of another type of cancer, acute or chronic inflammation, infection, respiratory or autoimmune disease, and those who refused to participate in the proposed study. This study was approved by the Research Ethics Committee of the Faculty of Medical Sciences of the State University of Campinas (FCM-Unicamp).

Quantification of serum SAA

SAA concentrations were determined in duplicate of serum samples by high-sensitivity (0.82 mg/L) microlatex agglutination tests, detected by nephelometry and conducted according to the manufacturer's recommendations (BN Prospec, SIEMENS).

Data analysis

Nonparametric tests were used because of lack of normal distribution. Anthropometric and continuous variables of the groups were compared by Mann–Whitney tests. The frequencies of categorical variables were analyzed by χ^2 or Fisher exact tests, and the correlation analyses were done by Spearman test. To compare the values of SAA among categorical variables, analysis of covariance (ANCOVA) was used after adjustment for BMI and waist circumference. Furthermore, multivariate linear regression analysis for SAA was conducted using a stepwise procedure to derive a final model of variables that had a significant independent relationship with SAA. These analyses included all covariates that were significant in univariate analysis, that is, age, time of menopause, BMI, waist circumference, and WHR. The variable SAA was rank-transformed before using it in ANCOVA or in linear regression analysis. All analyses were conducted with SPSS16.0 software (SPSS) and the P value of 0.05 or less was considered significant.

Results

Characteristics of postmenopausal patients bearing breast cancer

As previously stated the patients enrolled in the present study were classified in 2 groups according to BMI plus abdominal fat absence or presence, based on waist circumference measurement. Demographic and anthropometric data of the patients are presented in Table 1. As expected, the 2 different groups showed significant differences in relation to BMI ($P < 0.0001$), waist circumference ($P < 0.0001$) and WHR ($P = 0.0028$).

SAA concentration is positively associated with obesity

We can observe in Table 2 that serum SAA concentration was significantly higher in overweight/obese

Table 1. Characteristics of postmenopausal women bearing breast cancer

	Overweight/obese ($n = 54$)	Nonobese ($n = 19$)
Age, y		
Median (IQR)	61 (55–67)	56 (51–65)
Time of menopause, y		
Median (IQR)	14 (6–20)	7 (3–17)
BMI, kg/m^2		
Median (IQR)	30.6 (28.4–33.3)[a]	22.6 (21.1–22.8)
WC, cm		
Median (IQR)	102 (94–109)[a]	84 (80–85)
WHR		
Median (IQR)	0.95 (0.91–0.98)[a]	0.89 (0.84–0.95)
Race/ethnicity[b]		
Caucasian (%)	100	100

NOTE: Overweight/obese: BMI $\geq$ 25 kg/m^2; WC $>$ 88 cm. Nonobese: BMI $\leq$ 24.9 kg/m^2; WC $\leq$ 88 cm. Data are expressed as median and interquartile range (IQR). Mann–Whitney test.

[a]$P < 0.05$. P value of 0.05 or less was considered significant.

[b]The study group is from the Southeast region in Brazil, where Caucasians are majority and descend mainly from diverse European ethnicities.

Published OnlineFirst January 8, 2013; DOI: 10.1158/1055-9965.EPI-12-1020

Table 2. SAA concentrations in patients with breast cancer grouped according to anthropometric or tumor characteristics

	SAA (mg/L)		
	n	**Median (IQR)**	***P***
Overweight/obese[a]	54	4.94 (2.75–7.18)	
Nonobese	19	2.66 (1.52–4.18)	0.008[c]
Stage I[b]	24	3.31 (2.56-5.95)	
Stage II	30	3.48 (2.01–6.77)	
Stage III	14	5.32 (1.73–19.10)	0.454
ER^+	55	3.30 (2.01–6.77)	
ER^-	11	5.28 (4.18–6.57)	0.033[d]
PR^+	38	2.99 (1.73–6.58)	
PR^-	28	4.26 (3.09–7.35)	0.079
$HER2^+$	23	4.18 (2.54–6.01)	
$HER2^-$	49	3.31 (2.40–7.04)	0.388

NOTE: Data are expressed as median (IQR).

[a]Overweight/obese: BMI $\geq$ 25 kg/m^2; WC > 88 cm. Nonobese: BMI $\leq$ 24.9 kg/m^2; WC $\leq$ 88 cm.

[b]TNM staging system or prognostic group.

[c]Mann–Whitney test for comparison between overweight/obese vs. nonobese group.

[d]ANCOVA adjusted for BMI and waist circumference for tumor characteristics groups. *P* value of 0.05 or less was considered significant.

than in nonobese patients. The different groups showed the following values of median and interquartile range (IQR): 4.94 mg/mL (2.71–7.18) in overweight/obese versus 2.66 mg/mL (1.52–4.18) in nonobese group ($P = 0.008$).

Afterward, when the total patient group was analyzed by the Spearman correlation test, we found that SAA levels were positively associated with BMI ($r = 0.37$; $P = 0.001$) and waist circumference ($r = 0.38$; $P = 0.0006$) as it is shown in Fig. 1. Furthermore, results from multivariate linear regression analysis for SAA, that were conducted using a stepwise criterion, showed that BMI plus waist circumference ($P = 0.01$), but not WHR, had a significant independent relationship with SAA, meaning that patients with the highest concentration of SAA are obese (highest BMI and waist circumference).

However, as shown in Fig. 1, there is a significant spread in SAA values among women with high BMI/WC, which can be explained by the lack of normal distribution of SAA values in the study population. Thus, before being used in linear regression analysis, the variable was rank-transformed to cope with the influence of outliers on the results. Five of 6 outliers had fasting glucose values above reference range and 4 of 5 also had high-density lipoprotein cholesterol (HDL-chol) concentrations below the reference range (data not shown).

Serum concentration of SAA among patients presenting different tumor characteristics

To compare SAA concentrations among patients bearing tumors with different characteristics, we conducted ANCOVA after adjustment for BMI and waist circumference. We can observe in Table 2 significant difference in SAA concentrations between patients presenting ER^- or ER^+ tumors. We found SAA median (IQR) values of 5.28 mg/mL (4.18–6.57) in patients bearing ER^- tumors versus 3.30 mg/mL (2.01–6.77) in ER^+ tumors ($P = 0.033$).

Discussion

In this study, we have shown that postmenopausal women bearing breast cancer showed serum SAA concentrations positively associated with BMI and waist circumference, meaning that patients with the highest concentrations of SAA are obese. Moreover, 5 of 6 outliers with the highest values of SAA had fasting glucose concentrations above the reference values and 4 of 5 had also HDL-chol concentrations below reference range. Although none of them had been diagnosed with insulin-dependent or type II diabetes, 4 of them met the

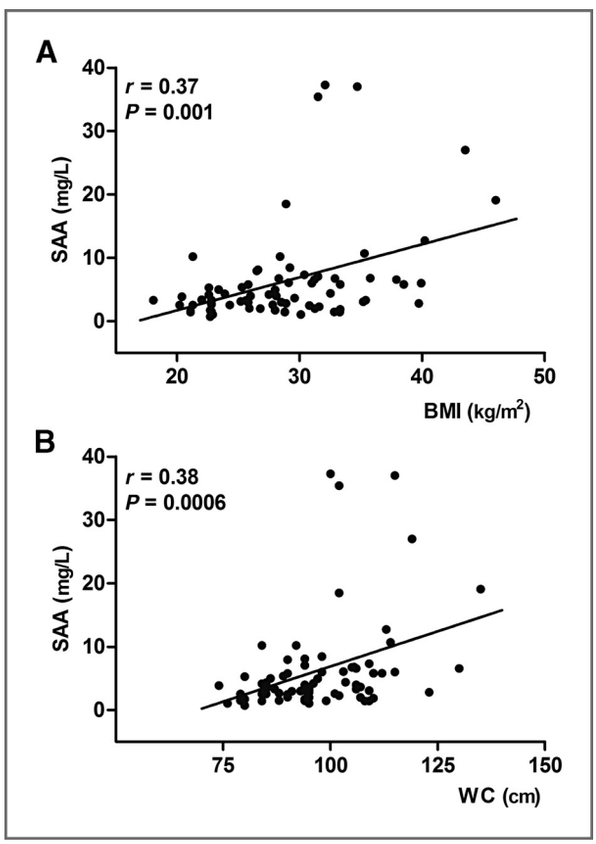

Figure 1. Association of SAA with BMI and waist circumference (WC). A, positive correlation with BMI ($r = 0.37$; $P = 0.001$). B, positive correlation with waist circumference ($r = 0.38$; $P = 0.0006$). Correlations carried by Spearman test. *P* value of 0.05 or less was considered significant.

Published OnlineFirst January 8, 2013; DOI: 10.1158/1055-9965.EPI-12-1020

criteria for metabolic syndrome (e.g., elevated fasting glucose, abdominal obesity, and reduced HDL), suggesting that SAA could be a link between obesity and its metabolic complications in those patients (14).

Nowadays, it is accepted that SAA along with other inflammatory proteins lead to the adipose tissue into a chronic inflammatory state, accompanied by the infiltration of immune cells, such as macrophages (18). Chronic inflammation is a contribution factor for the development and the promotion of carcinogenesis by complex processes, such as infiltration of macrophages into premalignant mammary tissue (19). Therefore, we could postulate an influence of obesity via SAA in breast cancer development. The fact that we found serum concentrations of SAA differentially elevated in overweight/obese patients leads us to suppose that the role of this protein in breast cancer development might be restrict to overweight/obese patients.

To constitute the nonobese group in this study, we used BMI plus waist circumference measurement that is an indirect estimate of central adiposity. This allowed comparison between groups, not only by the differences in BMI, but also by the indirect characterization of the presence or absence of visceral adiposity. The importance of this approach is corroborated by Lee and colleagues, who showed that healthy women in the transition from pre- to postmenopausal status, had an enhancement in the intra-abdominal fat depot, and that the increase was positively correlated with changes in several adipokines, including SAA (20). In addition, a more recent study shows the association of elevated concentration of SAA with increased adiposity in breast cancer survivors (21), emphasizing that adiposity as measured by dual energy X-ray absorptiometry (DEXA) is a strong predictor of SAA level.

We have not found correlation between serum SAA levels and clinicopathologic staging of tumors, but we found higher SAA concentrations in women bearing ER^- tumors than in ER^+ tumor counterparts.

Although the last result is in agreement with Pierce and colleagues that observed slightly stronger associations of SAA with ER^- and ER^-/PR^- strata (16), one would consider an unexpected result, as inflammatory factors have been indirectly associated with higher circulating estrogen level, as well as with ER expression in postmenopausal women presenting breast cancer (3).

The cross-sectional nature of our study does not allow inferences beyond the association perspective. However, considering the previously suggested role of SAA in the worse prognosis of breast cancer (16), one might consider its action in the mechanism of ER downregulation, as ER^-breast cancers are usually more aggressive and have poor prognosis. Recently, Ryu and colleagues showed that hypoxia-inducible factor-1α (HIF-1α) represses ER-α gene transcription in MCF-7 and T47D human breast cancer cells (22). Therefore, considering that cytokines such as TNF-α can stimulate HIF-1α production (23) and that SAA can stimulate TNF-α synthesis in different cell types (14, 24), it would be reasonable to hypothesize that in the tumor microenvironment enriched with inflammatory factors (e.g., SAA, TNF-α, etc.), from circulation or local cells (e.g., mammary adipocyte, endothelial, or migrated immune cells), it might have an upregulation of HIF-1α production that, consecutively, would repress ER expression. However, further mechanistic studies certainly will be needed to verify this hypothesis.

To our knowledge, we have shown for the first time in an untreated postmenopausal group of patients with breast cancer, that high serum SAA levels are obesity-dependent (BMI and waist circumference). Thus, we might suggest that obesity, via SAA, could influence the development and the progression of breast cancer in postmenopausal women. In line with this assumption, we agree with Dee and colleagues that postulate that treating obesity would curb inflammatory state that, in turn, would confer a better prognosis for patients with breast cancer (21).

The main limitation of this study is its small sample size. It is a consequence from our primary approach—breast cancer and obesity in postmenopausal—and also from the strict selection criteria used, mainly to constitute the nonobese group. However, nonparametric statistical tests were used to compensate for the small sample size. Besides, the sample is representative of the most developed region in Brazil—the Southeast region, which has the highest incidence of breast cancer in the country (25).

In this study, it was not possible to correlate serum SAA with poor prognosis. This could be justified by the homogeneity of the clinicopathologic characteristics of tumors of the patients studied, possibly because they were bearing primary tumors and/or would be due to early diagnosis.

A prospective study of this population to assess SAA concentration at the follow-up, overall, and disease-free survival is in course and should bring contribution to evaluate the clinical role of SAA in breast cancer in the context of obesity.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: S. de Barros-Mazon
Development of methodology: A.B. Santana
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.B. Santana, M.S.C. Gurgel, J.F.O. Montanari, F. M. Bonini
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.B. Santana, M.S.C. Gurgel, S. de Barros-Mazon
Writing, review, and/or revision of the manuscript: A.B. Santana, M.S.C. Gurgel, S. de Barros-Mazon
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.B. Santana, J.F.O. Montanari, F.M. Bonini
Study supervision: S. de Barros-Mazon

Acknowledgments

The authors thank H.C. Machado and C.A.M. Silva for statistical analyses, as well as S.O.A. Teixeira and A.C.E. Dias for technical assistance.

Published OnlineFirst January 8, 2013; DOI: 10.1158/1055-9965.EPI-12-1020

Grant Support

This study was supported by State of São Paulo Research Foundation (Fapesp), #09/17084-7 and State University of Campinas Teaching and Research Support Foundation (Faepex/Unicamp) for de Barros-Mazon, S. Brazil. Coordination for the Improvement of Higher Education Personnel (Capes): MSc fellowship (to A.B. Santana). Institutional Program of Scientific Initiation Scholarships/National Counsel of Technological and Scientific Development (PIBIC/CNPq): SCI scholarship (to F.M. Bonini).

Received September 7, 2012; revised November 20, 2012; accepted December 11, 2012; published OnlineFirst January 8, 2013.

References

1. Globocan 2008. Cancer incidence and mortality worldwide. Lyon, France: IARC; 2008 [accessed 2011 Nov 24]. Available from: http://globocan.iarc.fr/factsheets/cancers/breast.asp.
2. Kuhl H. Breast cancer risk in the WHI study: the problem of obesity. Maturitas 2005;51:83–97.
3. Lorincz AM, Sukumar S. Molecular links between obesity and breast cancer. Endocr Relat Cancer 2006;13:279–92.
4. Siiteri PK. Adipose tissue as a source of hormones. Am J Clin Nutr 1987;45:277–82.
5. Cleary MP, Grossmann ME. Minireview: obesity and breast cancer: the estrogen connection. Endocrinology 2009;150:2537–42.
6. Vona–Davis L, Rose PR. Adipokines as endocrine, paracrine, and autocrine factors in breast cancer risk and progression. Endocr Relat Cancer 2007;14:189–206.
7. Trayhurn P, Wood IS. Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 2004;92:347–55.
8. de Barros-Mazon S, Marin DM, Carvalho CP, Alegre SM. Inflammatory and metabolic markers in pre- and post-treatment of obesity. In: Atta-ur-Rahman FRS, Iqbal Choudhary, editors. Anti-obesity drug discovery and development. Oak Park, IL: Bentham Science Publishers Ltd.; 2011. p. 49–66.
9. Blann AD, Byrne GJ, Baildm AD. Increased soluble intercellular adhesion molecule-1, breast cancer and the acute phase response. Blood Coagul Fibrinolysis 2002;13:165–8.
10. O'Hanlon DM, Lynch J, Cormican M, Given HF. The acute phase response in breast carcinoma. Anticancer Res 2002;22:1289–93.
11. Lithgow D, Covington C. Chronic inflammation and breast pathology: a theoretical model. Biol Res Nursing 2005;7:118–29.
12. Schultz DR, Arnold PI. Properties of four acute phase proteins: C-reactive, serum amyloid A protein, alpha 1-caid glycoprotein, and fibrinogen. Semin Arthritis Reum 1990;20:129–47.
13. Ulhar CM, Whitehead A. Serum amyloid A, the major vertebrate acute phase reactant. Eur J Biochem 1999;265:501–23.
14. Yang RZ, Lee MJ, Hu H, Pollin TI, Ryan AS, Nicklas BJ, et al. Acute-phase serum amyloid A: an inflammatory adipokine and potential link between obesity and its metabolic complications. PLoS Med 2006;3:e287.
15. Poitou C, Divoux A, Faty A, Tordjman J, Hugol D, Aissat A, et al. Role of serum amyloid A in adipocyte-macrophage cross talk and adipocyte cholesterol efflux. J Clin Endocrinol Metab 2009;94:1810–7.
16. Pierce BL, Ballard-Barbash R, Bernstein L, Baumgartner RN, Neuhouser ML, Wener KB, et al. Elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients. J Clin Oncol 2009;27:3437–44.
17. World Health Organization. Global database on body mass index; 2009 [accessed 2009 Nov 25]. Available from: http://apps.who.int/bmi.
18. Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444:860–7.
19. Schwertfeger KL, Xian W, Kaplan AM, Burnett SH, Cohen DA, Rosen JM. A critical role for the inflammatory response in a mouse model of preneoplastic progression. Cancer Res 2006;66:5676–85.
20. Lee CG, Carr MC, Murdoch SJ, Mitchell E, Woods NF, Wener MH, et al. Adipokines, inflammation, and visceral adiposity across the menopausal transition: a prospective study. J Clin Endocrinol Metab 2009;94:1104–10.
21. Dee A, McKean-Cowdin R, Neuhouser ML, Ulrich CM, Baumgartner RN, McTiernan A, et al. DEXA measures of body fat percentage and acute phase proteins among breast cancer survivors: a cross-sectional analysis. BMC Cancer 2012;12:343.
22. Ryu K, Park C, Lee Y. Hypoxia-inducible factor 1 alpha represses the transcription of the estrogen receptor alpha gene in human breast cancer cells. Biochem Biophys Res Commun 2011;407:831–6.
23. Bilton RL, Booker GW. The subtle side to hypoxia inducible factor (HIFalpha) regulation. Eur J Biochem 2003;270:791–8.
24. Hatanaka E, Monteagudo PT, Marrocos MS, Campa A. Interaction between serum amyloid A and leukocytes—a possible role in the progression of vascular complications in diabetes. Immunol Lett 2007;108:160–6.
25. Incidência de Câncer no Brasil. Síntese de resultados e comentários; 2012 [accessed 2012 Nov 12]. Available from: http://www.inca.gov.br/estimativa/2012/index.asp.

Published OnlineFirst January 8, 2013; DOI: 10.1158/1055-9965.EPI-12-1020

Cancer Epidemiology, Biomarkers & Prevention

AACR American Association for Cancer Research

Serum Amyloid A Is Associated with Obesity and Estrogen Receptor–Negative Tumors in Postmenopausal Women with Breast Cancer

Aline Barros Santana, Maria Salete Costa Gurgel, Joelma Ferreira de Oliveira Montanari, et al.

Cancer Epidemiol Biomarkers Prev 2013;22:270-274. Published OnlineFirst January 8, 2013.