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Plasma hPG_{80} (Circulating Progastrin) as a Novel Prognostic Biomarker for early-stage breast cancer in a breast cancer cohort

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Abstract

Background Recurrence and metastases are still frequent outcomes after initial tumour control in women diagnosed with breast cancer. Although therapies are selected based on tumour characteristics measured at baseline, prognostic biomarkers can identify those at risk of poor outcomes. Circulating progastrin or hPG_{80} was found to be associated with survival outcomes in renal and hepatocellular carcinomas and was a plausible prognostic biomarker for breast cancer.

Methods Women with incident breast cancers from Calgary, Alberta, Canada enrolled in the Breast to Bone (B2B) study between 2010 to 2016 and provided blood samples prior to any treatment initiation. Plasma from these baseline samples were analysed for circulating progastrin or hPG_{80}. Participant characteristics as well as tumour ones were evaluated for their association with hPG_{80} and survival outcomes (time to recurrence, recurrence – free survival, breast cancer specific survival and overall survival) in Cox proportional hazards regression models.

Results The 464 participants with measurable hPG_{80} in this study had an average age of 57.03 years (standard deviation of 11.17 years) and were predominantly diagnosed with Stage I (52.2%) and Stage II (40.1%) disease. A total of 50 recurrences and 50 deaths were recorded as of June 2022. In Cox PH regression models adjusted for chemotherapy, radiation therapy, cancer stage and age at diagnosis, log hPG_{80} (pmol/L) significantly increased the risks for recurrence (Hazard Ratio (HR) = 1.330, 95% Confidence Interval (CI) = (0.995 – 1.777, $p = 0.054$)), recurrence-free survival (HR = 1.399, 95% CI = (1.106 – 1.770), $p = 0.005$) and overall survival (HR = 1.385, 95% CI = (1.046 – 1.834), = 0.023) but not for breast cancer specific survival (HR = 1.015, 95% CI = (0.684 – 1.505), $p = 0.942$).

Conclusions hPG_{80} levels measured at diagnosis were significantly associated with the risk of recurrence or death from any cause in women with breast cancer. Since the recurrence rates of breast cancer are still relatively high amongst women diagnosed at an early stage, identifying women at high risk of recurrence at their time of diagnosis is important. hPG_{80} is a promising new prognostic biomarker that could improve the identification of women at higher risk of poor outcomes.

Keywords Breast cancer, Circulating progastrin, hPG_{80}, Prognostic biomarker, Recurrence, Survival

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Background

Breast cancer is not only the most common cancer amongst Canadian women, but is also the second leading cause of cancer-related deaths in Canadian women as well [1]. The 5-year net survival rates for women with breast cancer is 89% depicting a generally favourable prognosis of breast cancer due to early detection from breast cancer screening and modern treatments [1]. Currently, appropriate treatments for breast cancer are determined by the use of predictive markers [2, 3]. Predictive biomarkers allow clinicians to determine the best course of treatment depending on the type of breast cancer the woman has [3]. The most common breast cancer biomarkers are estrogen receptors (ER), progesterone receptors (PR), and Human Epidermal Growth Factor Receptor 2 (HER2) [2]. After determination of which receptors are positive, the breast cancer subtype is identified which affects the treatment options [2].

Prognostic biomarkers can supplement this information from predictive biomarkers and provide additional insights to clinicians on which course of treatment is most appropriate in the long term [3, 4]. Prognostic biomarkers indicate the aggressiveness, invasiveness, and extent of spread of tumors which can aid in determining the recurrence risks and even survival outcomes [3]. Traditional prognostic biomarkers include axillary lymph node status, tumor size and grade, age at diagnosis, and nuclear and histological grade [3, 4]. The most common prognostic blood-biomarkers used for breast cancer include cancer antigen 15–3 (CA 15–3), carcinoembryonic antigen (CEA), HER2, and mucin 1 [5]. A systemic review revealed that while these prognostic biomarkers are useful in the entire breast cancer population, their performance was suboptimal in young and elderly patient groups [4]. The combined use of predictive and prognostic biomarkers enables clinicians to select more individualized treatments for breast cancer patients. With increasing knowledge on the pathophysiology of breast cancer, identifying biomarkers that can provide more individualized and targeted therapy for women with breast cancer are still needed, especially those identifying aggressive disease.

Although earlier stages of breast cancer have a more favourable prognosis [6], approximately 20–30% of early stage breast cancer patients develop bone metastasis which is the most frequent site (about 70%) for breast cancer metastasis [7]. Local recurrence of breast cancer occurs in 8% to 10% of patients [8]. Unfortunately, bone metastasis is not curable with women experiencing bone-only metastasis having a median overall survival ranging from 3–5 years [9, 10]. The Canadian Cancer Society statistics suggest that of 78 Canadian women diagnosed with breast cancer, 15 will die from it [1]. Thus, identifying women at risk of disease recurrence, metastases or death at the time of diagnosis are needed to improve these outcomes.

A novel blood-based biomarker that has shown promise in several different types of cancer is circulating progastrin or hPG_{80}. It has been detected at significantly higher concentrations in the blood of cancer patients than in healthy blood donors [11, 12]. In physiology, progastrin is the precursor of gastrin synthetized by antrum G cells and processed into gastrin [13]. As a consequence, progastrin is barely detectable in the blood of healthy subjects [14]. hPG_{80} was initially studied in colorectal cancer and was found to be released in the blood stream from tumor cells, promoting carcinogenic activities [11, 14, 15]. In tumor cells, the *GAST* gene, which encodes hPG_{80}, is a direct target gene of the WNT/β-catenin oncogenic pathway which is activated in many cancers [16]. hPG_{80} is directly associated with tumor cells survival [11, 15, 17, 18]. More recently, in both renal and hepatocellular cancers, hPG_{80} has shown a significant association with survival for both cancers [19, 20]. The hepatocellular cancer patients showed a higher sensitivity to hPG_{80} than alpha-fetoprotein (AFP) – the standard available diagnostic marker for hepatocellular cancer [20]. Thus, the plausibility of utilizing hPG_{80} as a blood prognostic biomarker for breast cancer exists.

Previous research has shown the clinical utility of hPG_{80} to detect patients at risk for poor survival outcomes, even amongst those with early-stage disease [20]. So, its utility in a breast cancer cohort established to identify prognostic biomarkers was a unique opportunity to evaluate it in this patient group. This study evaluated the association of hPG_{80} with a variety of outcomes including disease recurrence, recurrence-free survival, breast cancer specific survival and overall survival among a cohort of breast cancer patients from the breast-to-bone (B2B) cohort.

Methods

Study population

The B2B Metastasis research program interviewed and recruited 478 women between 2010 to 2016 who met the eligibility criteria of having incident primary breast cancer (stage I-IIIc) at baseline, with no prior history of cancer (except for cervical in-situ neoplasia and non-melanoma skin cancer), who were between the ages 18 to 80 years and were residents of Calgary, Alberta, Canada and the surrounding areas. After diagnosis but before surgery or any treatments began, 471 participants provided blood samples of sufficient quantity and quality at a provincial laboratory location. Samples were transferred to the Alberta Cancer Research Biorepository (ACRB) for storage in -80 °C freezers. Patients were also followed-up

at 24, 48, and 72-month intervals post-diagnosis and were asked to complete self-administered follow-up Health and Lifestyle questionnaires, Canadian Diet History Questionnaires I/II, and Past Year Physical Activity Questionnaire; blood samples were also collected at these time points. Further information on the study population and recruitment can be found in the baseline paper [21].

hPG_{80}, participant and clinical variables

A 500 µl aliquot of EDTA plasma from 471 participants was retrieved from the ACRB inventory and were couriered on dry ice to the biology and pathology center (Les Hospices Civils de Lyon, France) on November 22, 2021 where the hPG_{80} levels were then measured using the $DxPG_{80}$ lab kit (Biodena care). The analytical performances of the kit are described in Cappellini et al. [22]. Briefly, the limit of detection (LoD) is 1 pmol/L and the limit of quantitation (LoQ) is 3.3 pmol/L. The inter- and intra-assay coefficients of variation (CV%) were below 10%. No cross-reactivity was detected with gastrin-17, Gastrin-Gly or CTFP (C-Terminus Flanking Peptide). No cross-reactivity was detected with other blood biomarkers such as CA125, CEA or PSA. No interference was detected with chemicals such as SN-38, 5-FU or triglycerides, cholesterol or hemoglobin [20]. hPG_{80} values for 464 participants were successfully obtained, although 129 of them had values below the assay's LoQ threshold.

A Health Records Technician with Alberta Health Services, the provincial health authority in Alberta, carried out chart reviews for 120 B2B participants identified to be at the highest or lowest risk of breast cancer recurrence using an algorithm based on administrative data [23]. Vital status was updated through linkage with the Alberta Cancer Registry (ACR), where dates and causes of death (if known) were obtained up to December 2021. Participant factors considered in this study included age at diagnosis and menopausal status recorded at baseline. Menopausal status was imputed for 35 for the 464 women who comprised this study population. Women older than 50 years of age or those who had a history of hysterectomy or oophorectomy at any age were deemed to be post-menopausal (22 women); the remaining ones were deemed to be pre-menopausal (13 women). Tumour characteristics measured at baseline included stage, grade, size and hormonal statuses. Treatment factors included chemotherapy, radiotherapy, immunotherapy and hormonal therapy. All participants had surgery to remove their tumours.

Statistical methods

Statistical methods included descriptive statistics of the clinical and demographic variables from the study population, including hPG_{80} levels. Survival events included breast cancer recurrence (local or distant), and death from any cause or from breast cancer. Survival outcomes were based on the time elapsed from diagnosis to the first event and included disease-free survival (first occurrence of death or recurrence), overall survival (death from any cause), breast cancer-specific survival (death from breast cancer), and time to breast cancer recurrence. The end date of June 2022 was used for censored observations. hPG_{80} levels below the threshold LoQ were imputed for 129 (out of 464) women in our study using a truncated Normal distribution via the R package *TruncNorm*. hPG_{80} values above the threshold LoQ were log transformed to generate a truncated Normal distribution that was used to generate log-transformed hPG_{80} values below the LoQ. These values were randomly assigned to the 129 women with missing values.

Clinically meaningful cut points of hPG_{80} levels that maximized each survival outcome were obtained using the R package *survminer*. Kaplan–Meier curves were generated for each survival outcome stratified by the low ($\leq$) or high ($>$) hPG_{80} group based on the respective hPG_{80} cut point. Adjustment for treatment (chemotherapy (Yes or No), radiotherapy (Yes or No), cancer stage (I, II, III) and age diagnosis (continuous scale) were included in Cox proportional hazards (PH) models for all survival outcomes that included log hPG_{80} levels measured on a continuous scale.

Menopausal status and age at diagnosis were assessed for their association with hPG_{80} including interactions. Sensitivity analyses were conducted by replacing age at diagnosis with menopausal status or a binary age at diagnosis; and hPG_{80} cut points replaced hPG_{80} measured on a continuous scale. Since this was a non-randomized study, cancer treatments (chemotherapy and radiation therapy) were forced in to provide a crude adjustment for their impact on survival outcomes. Cancer stage was the most significant tumour characteristic, had women with events at each level and never violated the proportional hazard (PH) assumption; it was also included in the final Cox PH models. Tumour grade and size did not meet at least one of these criteria so were not included. Proportional hazards assumptions were tested by assessing Schoenfeld residuals and Harrell's C calculated to assess the final model prediction. Statistical significance was set at 0.05 for all statistical analyses. All analyses were conducted using Stata 17.

Results

The 464 participants in this study were predominantly over 50 years of age (73.5%) and post-menopausal (75.4%). Most were diagnosed as a Luminal A subtype, as most had positive receptor status for estrogen (84.9%) and progesterone (78.2%), and negative receptor

status for HER-2 (78.2%). Participants were approximately evenly split on participation in chemotherapy (49.3% did not participate, while 50.7% did participate), but were more likely than not to have undergone radiation therapy (65.3%), hormone therapy (75.9%), and surgery (100.0%), while being less likely to have undergone immunotherapy (8.41%). The most frequent stage at diagnosis was Stage I (52.2%) followed by Stage II (40.1%). Tumours were more likely to be graded as medium (41.2%) or high (40.3%), with an average tumour size of 20.8 mm (Standard Deviation (SD) = 14.3 mm). At the study end date, 50 women had experienced a recurrence of their breast cancer and 50 had died, including 29 who had a previous recurrence. Table 1 contains descriptive statistics relevant to our analytical population.

Table 2 contains the summary of hPG_{80} cut point determinations found using the R package *survminer*. The same cut point value of 9.84 pmol/L was found for both recurrence-free survival ($p = 0.009$) and overall survival ($p < 0.001$), both of which were determined to be statistically significant using the Log-Rank test. A cut point of 6.77 pmol/L was found for breast cancer-specific survival ($p = 0.183$), and a cut point of 4.02 pmol/L was found for time to recurrence ($p = 0.240$), though neither were found to be statistically significant. Figure 1 contains the plotted Kaplan–Meier curves associated with these estimated hPG_{80} cut points for each survival outcome. The log rank tests were statistically significant for both time-to-recurrence (0.009) and overall survival (< 0.001) but not breast-cancer survival (0.183) or recurrence-free outcomes (0.240).

After adjusting the Cox PH models for age at diagnosis, participation in chemotherapy, participation in radiation therapy, and cancer stage, hPG_{80} was estimated to be a significantly hazardous predictor for recurrence-free survival (HR: 1.399; 95% Confidence Interval (CI): 1.106 – 1.770; $p = 0.005$) and overall survival (hazard ratio (HR): 1.385; 95% CI: 1.046 – 1.834; $p = 0.023$). Additionally, hPG_{80} was also estimated to be a potentially hazard predictor for time to recurrence (HR: 1.330; 95% CI: 0.995 – 1.777; $p = 0.054$) but was not found to be a significant predictor for breast cancer-specific survival (HR: 1.015; 95% CI: 0.684 – 1.505; $p = 0.942$). Harrell's C range from 0.64 to 070 indicating fair prediction based on these models (Table 3).

Additionally, age at diagnosis was found to be significantly protective predictor for time to recurrence (HR: 0.967; 95% CI: 0.940 – 0.996; $p = 0.027$). Cancer stage was found to be a significant hazardous predictor for all 4 survival outcomes; patients diagnosed with stage III breast cancers were estimated to be at much greater risk than patients diagnosed with stage I cancers in recurrence-free

Table 1 Descriptive Statistics for Study Population ($N = 464$)

Variable	Values
	Means (SD[a])
hPG_{80} (pmol/L)	6.05 (27.88)
Log- hPG_{80} (pmol/L)	0.98 (1.03)
Diagnosis Age (years)	57.04 (11.13)
Tumour Size (mm)	20.76 (14.32)
	Frequency (percentage)
Diagnosis Age Group (years)	
Under-50	123 (26.51)
50 +	341 (73.49)
Menopausal Status	
Pre-Menopausal	114 (24.57)
Post-Menopausal	350 (75.43)
Vital Status	
Alive	414 (89.22)
Deceased	50 (10.78)
Cause: Breast cancer	31 (62.00)
Cause: Other cancer	9 (18.00)
Cause: Other	10 (20.00)
Estrogen Receptor	
Positive	394 (84.91)
Negative	56 (12.07)
Missing	14 (3.02)
Progesterone Receptor	
Positive	363 (78.23)
Negative	86 (18.53)
Missing	15 (3.23)
HER-2 Receptor	
Positive	82 (17.67)
Negative	363 (78.23)
Missing	19 (4.09)
Hormone Receptor Combinations	
Triple Negative	30 (6.47)
HR[b] Negative, HER-2 Positive	17 (3.79)
HR Positive, HER-2 Negative	330 (71.12)
HR Positive, HER-2 Positive	63 (13.58)
Missing	24 (5.17)
Treatment Participation	
Chemotherapy	
Yes	229 (49.35)
No	235 (50.65)
Radiotherapy	
Yes	303 (65.30)
No	161 (34.70)
Surgery	
Yes	464 (100.00)
Hormone Therapy	
Yes	352 (75.86)
No	112 (24.14)

Table 1 (continued)

Variable	Values
Immunotherapy	
Yes	39 (8.41)
No	425 (91.59)
Cancer Stage	
I	242 (52.16)
II	186 (40.09)
III	36 (7.76)
Tumour Grade	
Low	71 (15.30)
Medium	191 (41.16)
High	187 (40.30)
Missing	15 (3.23)

[a] *SD* standard deviation

[b] *HR* hormone receptor; is negative if both Estrogen Receptor (ER) and Progesterone Receptor (PR) are negative; is positive if at least one of ER or PR is positive

survival (HR: 3.98; 95% CI: 1.73 – 9.17; $p=0.001$), overall survival (HR: 5.004; 95% CI: 1.93 – 12.95; $p=0.001$), breast cancer-specific survival (HR: 5.56; 95% CI: 1.87 – 16.54; $p=0.002$), and time to recurrence (HR: 3.37; 95% CI: 1.31 – 8.66; $p=0.011$).

The sensitivity analyses did not alter any of these findings for the final Cox PH model (results not shown). For instance, adding tumour grade to the base model (with or without stage) did not change the significance of hPG_{80} for each outcome. Similarly, substituting the continuous version of hPG_{80} with one based on outcome-specific cut points did not change the significance of hPG_{80} for each outcome. Adding breast cancer recurrence as time-varying factor in the overall survival model resulted in hPG_{80} having a slightly larger *p*-value of 0.066. The only exception was when menopausal status was substituted for age at diagnosis in the final model, it was statistically significant for recurrence-free and overall survival but not for time to recurrence.

Discussion

Our study found significant associations between increasing hPG_{80} levels measured at diagnosis and the risk of recurrence or death in women with breast cancer. This risk was independent of chemotherapy or radiation therapy received, age at diagnosis or stage of disease. Older women at diagnosis had lower risks of recurrence and women diagnosed at Stage III were at substantially higher risk of all four outcomes. Additionally, cancer stage was found to be a significant predictor of increased hazard for all three outcomes – stage III in particular, while age at diagnosis was a significant predictor of decreased hazard for time-to-recurrence. Using the clinically-relevant cut point versions of hPG_{80} did not change the conclusions that were based on the continuous version.

A recent paper compared hPG_{80} blood levels from 11 different cancers to hPG_{80} levels from healthy controls [11]. The median hPG_{80} levels were significantly higher in the blood of cancer patients (4.88 pmol/L) than healthy blood donors (1.05 pmol/L) [11]. The results of our research also support the relationship of increased hPG_{80} levels and more cancer-related outcomes. For instance, the proportion of recurrences in the low hPG_{80} group was 16.3% versus 27.5% in the high hPG_{80} group (data not shown). The clinical cut point identified in our study for time to recurrence (4.02 pmol/L) was similar to those found in renal cell and hepatocellular carcinoma patients [19, 20]. The other cut points for the remaining three survival outcomes were higher. The study on renal cell carcinoma and hepatocellular carcinoma showed a significant association with median overall survival where the cut-off for hPG_{80} level was at 4.5 pmol/L for an approximate 12 month survival for both cancers; higher levels of hPG_{80} resulted in shorter survival [19, 20]. Additionally, the study on hepatocellular cancer suggest that the cohort exhibited a higher sensitivity to hPG_{80} than AFP, the common prognostic biomarker used for hepatocellular carcinoma, where combined measurement of hPG_{80} and AFP (clinical cut-point at 100 ng/mL) improved prognosis for patients with low AFP [20].

The hPG_{80} gene is the direct target of the WNT/β-catenin pathway – a pathway involved in the tumorigenesis of multiple organs [24]. The WNT/β-catenin pathway is associated with pluripotency, self-renewal of stem cells, and differentiation; however abnormal activation of the pathway promotes activation of cancer stem

Table 2 Summary of hPG_{80} Cut point Determinations for each Survival Outcome ($N=464$)

Survival Outcome	hPG_{80} Cut point (pmol/L)	Log- hPG_{80} Cut point (pmol/L)	Log Rank Test Statistic (*p*-value)	N. Low Group[a]	N. High Group[b]
Recurrence-free survival	9.84	2.286	2.526 (0.009)	413	51
Overall survival	9.84	2.286	3.216 (< 0.001)	413	51
Breast-specific survival	6.77	1.913	1.263 (0.183)	389	75
Time-to-recurrence	4.02	1.391	1.165 (0.240)	336	128

[a] N. Low Group – Number of Participants below or equal to hPG_{80} cut point

[b] N. High Group – Number of Participants above hPG_{80} cut point

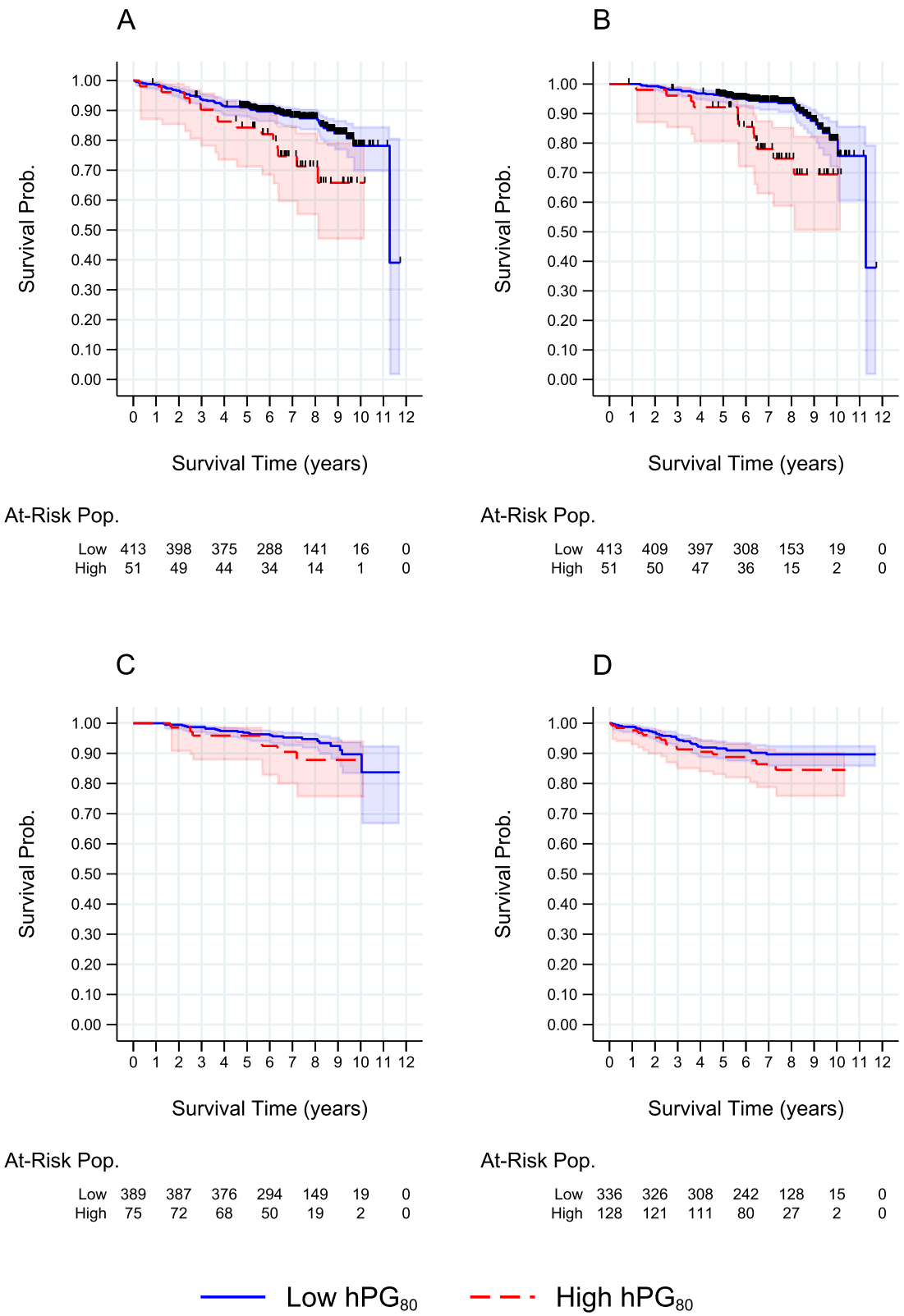

Fig. 1 Kaplan–Meier Curves for Survival Outcomes by hPG_{80} Group Status based on Corresponding Cut Point Determination. Censored observations denoted by '+', low hPG_{80} group is blue solid line, high hPG_{80} group is red dashed line. **a** Relapse-free survival, **b** Overall survival, **c** Breast – specific survival, and (**d**) Time – to – recurrence

Table 3 Cox Proportional Hazards Regression Model Results by Survival Outcome

Variable	Recurrence-Free Survival HR (*p*-value) 95% CI	Overall Survival HR (*p*-value) 95% CI	Breast-Specific Survival HR (*p*-value) 95% CI	Time to Recurrence HR (*p*-value) 95% CI
Log- hPG_{80} (pmol/L)	1.399 **(0.005)** **1.106 – 1.770**	1.385 **(0.023)** **1.046 – 1.834**	1.015 (0.942) 0.684 – 1.505	1.330 **(0.054)** **0.995 – 1.777**
Participation in Chemotherapy				
No	REFERENCE GROUP			
Yes	0.885 (0.717) 0.457 – 1.714	1.151(0.729) 0.521 – 2.541	1.630 (0.356) 0.578 – 4.600	1.158 (0.718) 0.523 – 2.565
Participation in Radiotherapy				
No	REFERENCE GROUP			
Yes	0.827 (0.477) 0.491 – 1.395	0.819 (0.540) 0.432 – 1.552	0.959 (0.926) 0.397 – 2.319	0.885 (0.707) 0.468 – 1.675
Diagnosis Age (years)	0.983 (0.171) 0.958 – 1.008	1.024 (0.148) 0.992 – 1.056	1.027 (0.191) 0.987 – 1.068	0.967 **(0.027)** **0.940 – 0.996**
Cancer Stage	Overall: ***p* = 0.005**	Overall: ***p* = 0.003**	Overall: ***p* = 0.001**	Overall: ***p* = 0.030**
Stage I	REFERENCE GROUP			
Stage II	1.599 (0.129) 0.872 – 2.931	1.452 (0.314) 0.703 – 3.003	1.000 (> 0.999) 0.378 – 2.645	1.352 (0.419) 0.651 – 2.804
Stage III	3.984 **(0.001)** **1.731 – 9.169**	5.004 **(0.001)** **1.933 – 12.952**	5.555 **(0.002)** **1.865 – 16.539**	3.373 **(0.011)** **1.314 – 8.655**
Harrell's C-Index	0.639	0.652	0.695	0.675

[1] Reported data: estimated hazard ratio and p-value (top); 95% confidence interval (bottom)

cell progression and hence, metastasis [25]. In colon carcinogenesis, the WNT/β-catenin pathway is further enhanced by excess hPG_{80} secretion and is considered an early marker of colon carcinogenesis [26]. Though the mechanism of the WNT/β-catenin pathway and hPG_{80} has primarily been studied in relation to colon cancer, similar mechanisms may also exist in other cancers, such as breast cancer.

Other novel biomarkers, still under evaluation in clinical studies, may also have the potential to determine prognostic outcomes of breast cancer. These potential biomarkers include circulating carcinoma proteins, circulating tumor cells, circulating cell-free tumor DNA, circulating microRNA (miRNA), extracellular vesicles, multi-analyte tests, and others [27]. The association between survival outcomes amongst some types of circulating carcinoma proteins are promising. For example, an increase in hepatocyte growth factors was shown in studies to be correlated with breast cancers as a risk factor and a high risk in metastatic progression [28]. However, interestingly, an increase in hepatocyte growth factors was paradoxically associated with long relapse-free survival [29]. In a systematic review and meta-analysis, circulating tumor DNA (ctDNA) was associated with a high risk of relapse; however, the review mentions there was heterogeneity of the studies, that all studies could not be included since relevant data were not available, and the studies used different techniques to quantify ctDNA [30]. Multiple types of circulating miRNA exist, and reviews suggest that high levels of circulating miRNA are associated with poor disease-free survival and prognosis of breast cancer [31]. Finally, a recent review suggests that extracellular vesicles are a potential biomarker for many cancers, including breast cancer [32]. Supporting this theory, one study on metastatic breast cancer patients found results suggesting extracellular vesicles are a potential predictor of progression free survival in metastatic breast cancer [33]. Compared to the above-mentioned new technologies, hPG_{80} is easily detectable in the plasma using ELISA technology and could be tested throughout the patient's journey to potentially identify patients who may need a deeper biological assessment at an acceptable economic cost.

The major strength of this cohort is that it is prospective, minimizing the possibility of selection bias and differential misclassification. Additionally, hPG_{80} was measured at baseline before treatment began so any potential treatment effects on the hPG_{80} levels were avoided. The results of this study could be generalizable to women diagnosed with breast cancer, as the underlying biological mechanism should be the same for all individuals; however, the cohort was primarily Caucasian, preventing the evaluation in other ethnic groups. Biologically, African-American women have a higher frequency of grade 3 tumors than Caucasian women, and higher proportions of triple negative breast cancers

[34]. Although the sample size for this study was nearly 500 women, very few women were diagnosed with a triple negative or HER-2 positive subtype limiting subgroup evaluations. Few women died from their breast cancer, likely contributing to low statistical power to detect an association with hPG_{80}. Despite this limitation, the cohort is able to provide novel and greater insight into an important relationship between hPG_{80} and breast cancer.

Conclusions

This is the first study observing the relationship between breast cancer outcomes and hPG_{80} levels. The prevalence of bone metastases in early stage breast cancers in Canada is 20–30%, providing a compelling need for better prognostic tools [7]. Future studies could focus on determining robust clinical cut points for all breast cancer subtypes, the provision of more customized treatments. Additionally, longitudinal measurements of hPG_{80} levels from diagnosis to events like recurrence or death could better elucidate this relationship.

Abbreviations

ACRB	Alberta Cancer Research Biorepository
AFP	Alpha-fetoprotein
B2B	Breast to Bone cohort
CA 15–3	Cancer antigen 15–3
CEA	Carcinoembryonic antigen
ctDNA	Circulating tumor DNA
EDTA	Ethylenediaminetetraacetic acid
ER	Estrogen receptor
Her 2	Human Epidermal Growth Factor Receptor 2
HR	Hazard ratio
LoD	Limit of detection
LoQ	Limit of quantitation
miRNA	MicroRNA
PH	Proportional hazards
PR	Progesterone receptor
SD	Standard Deviation

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Authors' contributions

AP and KK conceived of the study, prepared the ethics application and signed the material transfer agreement with Alberta Health Services; AP arranged the shipping of the plasma samples to France, the running of the DxPG80 lab kit at the Lyon Sud Hospital and then sending the hPG80 results for statistical analysis to KK; AH carried out the statistical analyses; AP, DJ, LP, KK, BV and AH provided the interpretation of data; MK drafted the manuscript; all authors contributed to the revisions of the manuscript, have approved this submitted version of the manuscript and agree to be personally accountable for their own contributions. They will also ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature. The author(s) read and approved the final manuscript.

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Availability of data and materials

The datasets analyzed during the current study are not publicly.available due to limitations on the participant consents obtained for enrollment in the B2B study. Please contact Karen Kopciuk to enquire about data availability.

Declarations

Ethics approval and consent to participate

Ethics approval for this study (HREBA.CC-19–0318) was granted from the Health Research Ethics Board of Alberta-Cancer Committee. Informed consent was obtained from all subjects involved in the Breast to Bone (B2B) study. This research project was carried out in accordance with relevant guidelines and regulations in the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

D.J. is the Senior Scientific Advisor of Biodena Care. A.P. is the Chief Scientific Officer of Biodena Care. B.V. is the Medical Science Liason at Biodena Care. The remaining authors declare no competing interests.

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References

1. Breast Cancer Statistics: Canadian Cancer Society; 2022 [updated May 2022]. Available from: https://cancer.ca/en/cancer-information/cancer-types/breast/statistics.
2. Gamble P, Jaroensri R, Wang H, Tan F, Moran M, Brown T, et al. Determining breast cancer biomarker status and associated morphological features using deep learning. Commun Med. 2021;1(1):14.
3. Taneja P, Maglic D, Kai F, Zhu S, Kendig RD, Fry EA, et al. Classical and Novel Prognostic Markers for Breast Cancer and their Clinical Significance. Clin Med Insights Oncol. 2010;4:15–34.
4. Phung MT, Tin Tin S, Elwood JM. Prognostic models for breast cancer: a systematic review. BMC Cancer. 2019;19(1):230.
5. Berghuis AMS, Koffijberg H, Prakash J, Terstappen LW, IJ MJ. Detecting Blood-Based Biomarkers in Metastatic Breast Cancer: A Systematic Review of Their Current Status and Clinical Utility. Int J Mol Sci. 2017;18((2):363.
6. Prognosis and survival for breast cancer: Canadian Cancer Society; 2022 [Available from: https://cancer.ca/en/cancer-information/cancer-types/breast/prognosis-and-survival#:~:text=The%20stage%20is%20the%20main,has%20a%20less%20favourable%20prognosis.
7. Kennecke H, Yerushalmi R, Woods R, Cheang MC, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol. 2010;28(20):3271–7.
8. Lafourcade A, His M, Baglietto L, Boutron-Ruault MC, Dossus L, Rondeau V. Factors associated with breast cancer recurrences or mortality and dynamic prediction of death using history of cancer recurrences: the French E3N cohort. BMC Cancer. 2018;18(1):171.
9. Lee SJ, Park S, Ahn HK, Yi JH, Cho EY, Sun JM, et al. Implications of bone-only metastases in breast cancer: favorable preference with excellent outcomes of hormone receptor positive breast cancer. Cancer Res Treat. 2011;43(2):89–95.
10. Ahn SG, Lee HM, Cho SH, Lee SA, Hwang SH, Jeong J, et al. Prognostic factors for patients with bone-only metastasis in breast cancer. Yonsei Med J. 2013;54(5):1168–77.
11. You B, Mercier F, Assenat E, Langlois-Jacques C, Glehen O, Soulé J, et al. The oncogenic and druggable hPG80 (Progastrin) is overexpressed in

multiple cancers and detected in the blood of patients. EBioMedicine. 2020;51: 102574.
12. Chauhan A, Prieur A, Kolesar J, Arnold S, Payen L, Mahi Y, et al. hPG(80) (Circulating Progastrin), a Novel Blood-Based Biomarker for Detection of Poorly Differentiated Neuroendocrine Carcinoma and Well Differentiated Neuroendocrine Tumors. Cancers (Basel). 2022;14(4):863.
13. Rehfeld JF, Zhu X, Norrbom C, Bundgaard JR, Johnsen AH, Nielsen JE, et al. Prohormone convertases 1/3 and 2 together orchestrate the site-specific cleavages of progastrin to release gastrin-34 and gastrin-17. Biochem J. 2008;415(1):35–43.
14. Siddheshwar RK, Gray JC, Kelly SB. Plasma levels of progastrin but not amidated gastrin or glycine extended gastrin are elevated in patients with colorectal carcinoma. Gut. 2001;48(1):47–52.
15. Prieur A, Cappellini M, Habif G, Lefranc M-P, Mazard T, Morency E, et al. Targeting the Wnt Pathway and Cancer Stem Cells with Anti-progastrin Humanized Antibodies as a Potential Treatment for K-RAS-Mutated Colorectal CancerAnti-progastrin Antibody for K-RAS–Mutated Colorectal Cancer. Clin Cancer Res. 2017;23(17):5267–80.
16. Koh TJ, Bulitta CJ, Fleming JV, Dockray GJ, Varro A, Wang TC. Gastrin is a target of the beta-catenin/TCF-4 growth-signaling pathway in a model of intestinal polyposis. J Clin Invest. 2000;106(4):533–9.
17. Pai SG, Carneiro BA, Mota JM, Costa R, Leite CA, Barroso-Sousa R, et al. Wnt/beta-catenin pathway: modulating anticancer immune response. J Hematol Oncol. 2017;10(1):101.
18. Pannequin J, Delaunay N, Buchert M, Surrel F, Bourgaux JF, Ryan J, et al. Beta-catenin/Tcf-4 inhibition after progastrin targeting reduces growth and drives differentiation of intestinal tumors. Gastroenterology. 2007;133(5):1554–68.
19. Kohli M, Tan W, Vire B, Liaud P, Blairvacq M, Berthier F, et al. Prognostic Value of Plasma hPG(80) (Circulating Progastrin) in Metastatic Renal Cell Carcinoma. Cancers (Basel). 2021;13(3).
20. Dupuy M, Iltache S, Rivière B, Prieur A, Pageaux GP, Bedoya JU, et al. Plasma hPG(80) (Circulating Progastrin) as a Novel Prognostic Biomarker for Hepatocellular Carcinoma. Cancers (Basel). 2022;14(2):402.
21. Brockton NT, Gill SJ, Laborge SL, Paterson AHG, Cook LS, Vogel HJ, et al. The Breast Cancer to Bone (B2B) Metastases Research Program: a multidisciplinary investigation of bone metastases from breast cancer. BMC Cancer. 2015;15(1):512.
22. Cappellini M, Flaceliere M, Saywell V, Soule J, Blanc E, Belouin F, et al. A novel method to detect hPG80 (human circulating progastrin) in the blood. Anal Methods. 2021;13(38):4468–77.
23. Xu Y, Kong S, Cheung WY, Bouchard-Fortier A, Dort JC, Quan H, et al. Development and validation of case-finding algorithms for recurrence of breast cancer using routinely collected administrative data. BMC Cancer. 2019;19(1):210.
24. Katoh M, Katoh M. Molecular genetics and targeted therapy of WNT-related human diseases (Review). Int J Mol Med. 2017;40(3):587–606.
25. Zhang Y, Wang X. Targeting the Wnt/β-catenin signaling pathway in cancer. J Hematol Oncol. 2020;13(1):165.
26. Tanaka H, Kawaguchi M, Shoda S, Miyoshi T, Iwasaki R, Hyodo F, et al. Nuclear Accumulation of β-Catenin in Cancer Stem Cell Radioresistance and Stemness in Human Colon Cancer. Anticancer Res. 2019;39(12):6575–83.
27. Li J, Guan X, Fan Z, Ching LM, Li Y, Wang X. Non-Invasive Biomarkers for Early Detection of Breast Cancer. Cancers (Basel). 2020;12(10):2767.
28. Owusu BY, Galemmo R, Janetka J, Klampfer L. Hepatocyte Growth Factor, a Key Tumor-Promoting Factor in the Tumor Microenvironment. Cancers (Basel). 2017;9(4):35.
29. Veyssière H, Bidet Y, Penault-Llorca F, Radosevic-Robin N, Durando X. Circulating proteins as predictive and prognostic biomarkers in breast cancer. Clin Proteomics. 2022;19(1):25.
30. Cullinane C, Fleming C, O'Leary DP, Hassan F, Kelly L, O'Sullivan MJ, et al. Association of Circulating Tumor DNA With Disease-Free Survival in Breast Cancer: A Systematic Review and Meta-analysis. JAMA Network Open. 2020;3(11):e2026921-e.
31. Cardinali B, Tasso R, Piccioli P, Ciferri MC, Quarto R, Del Mastro L. Circulating miRNAs in Breast Cancer Diagnosis and Prognosis. Cancers (Basel). 2022;14(9):2317.
32. St-Denis-Bissonnette F, Khoury R, Mediratta K, El-Sahli S, Wang L, Lavoie JR. Applications of Extracellular Vesicles in Triple-Negative Breast Cancer. Cancers (Basel). 2022;14(2):451.
33. Tian F, Zhang S, Liu C, Han Z, Liu Y, Deng J, et al. Protein analysis of extracellular vesicles to monitor and predict therapeutic response in metastatic breast cancer. Nat Commun. 2021;12(1):2536.
34. Jemal A, Robbins AS, Lin CC, Flanders WD, DeSantis CE, Ward EM, et al. Factors That Contributed to Black-White Disparities in Survival Among Nonelderly Women With Breast Cancer Between 2004 and 2013. J Clin Oncol. 2018;36(1):14–24.

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