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Role of the serum levels of the inter-organs messenger fibroblast growth factor 21 (FGF21) in the diagnosis and prognosis of breast cancer patients

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Abstract

FGF21 regulates local and systemic metabolic homeostasis. High serum FGF21 was found in obesity, metabolic syndrome, type 2 diabetes mellitus, and coronary heart disease. The pathways linking obesity and breast cancer remain elusive. We aimed to analyze the serum FGF21 in breast cancer patients at diagnosis. Circulating FGF21 levels in 45 breast cancer women (median age 59, range 32–88 years) and 51 age-matched healthy controls were evaluated using a quantitative ELISA assay. Patients' samples were obtained before surgery ahead of any previous therapy. Breast cancer patients showed significantly elevated serum FGF21 (median 267.13, range 28.41-780.45) respect to healthy controls (76.86, 0.00-425.60) ($p < 0.0001$). A ROC curve determined a cut-off value of 130.64 pg/ml to define positive or high FGF21 levels. Based on this cut-off point, 30/45 (66.7%) breast cancer patients showed positive serum FGF21 levels as compared to 18/51 (35.3%) healthy controls. Circulating FGF21 levels could be useful as a highly sensitive diagnosis biomarker for early breast cancer detection. We did not find any significant association between the serum FGF21 levels, and many clinical-pathological or metabolic parameters determined at the diagnosis of the primary disease. Interestingly, a statistically significant correlation was determined between serum FGF21 and the body mass index (BMI). Furthermore, patients with positive FGF21 serum levels had a worst overall survival (Log Rank Test [Mantle Cox] $p = 0.017$). We propose serum FGF21 levels determined at the diagnosis of primary breast cancer as a promising diagnostic and prognosis biomarker in this oncological disease.

Keywords Serum fibroblast growth factor 21, Breast Cancer, Obesity, Metabolic syndrome, Diagnosis biomarker, Prognosis Biomarker.

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Introduction

Breast cancer research and therapies have been greatly advanced over the years and nowadays, based on the breast cancer subtype and stage, the therapeutic strategies have improved the patient's survival. Nevertheless, breast cancer is a disease with a diverse genetic and molecular heterogeneity which makes it the most diagnosed cancer worldwide and the deadliest cancer in women [1–5]. Moreover, higher breast cancer mortality is observed in obese breast cancer patients [6].

Obesity which is an epidemic and has steadily increased over the last decades; has a rising prevalence in women. Epidemiological studies have suggested a close link between the local and systemic effects of obesity and an increased risk of developing several types of cancers, including endometrial, colorectal, and breast cancer. To date, despite important advances in the past, our understanding of the molecular mechanisms of obesity- breast cancer link remains incomplete. There is an urgent need to investigate the potential pathways linking obesity and breast cancer to have an early diagnosis in patients and optimize the chance of cure. The finding of new biomarkers is needed for breast cancer as well as reliable predictors of outcome and therapy response [7–16].

Fibroblast growth factor 21 (FGF21) is a protein produced mainly in the liver and regulated by peroxisome proliferator-activated receptor-α (PPAR-α) which functions in lipid metabolism. However, more data support a versatile role for this molecule acting as a physiologic alert system in several diseases. The elucidation of the endocrine FGF21 signaling pathways in the control of metabolic disease, energy homeostasis and their complications has raised great interest and hope in the last years [17, 18].

FGF21 belongs to a FGF sub family which acts as an endocrine factor. It regulates local and systemic metabolic homeostasis of lipid, glucose and energy metabolism. FGF21 acts as a stress-responsive factor, being its expression induced in response to diverse metabolic and cellular physiological or pathological stressors [19]. It has been reported that FGF21 mediates the adaptive starvation response to induce ketogenesis, gluconeogenesis, lipolysis, and lipid β-oxidation [20, 21]. The liver is the major metabolic organ producing FGF21 while the white adipose tissue is also contributing to the metabolic effects of FGF21. Interestingly, this inter-organ crosstalk messenger is also expressed in the pancreas, skeletal muscle, brown adipose tissue (BAT), heart, and brain [22].

High serum levels of FGF21 were found in obese individuals, subjects with metabolic syndrome, type 2 diabetes mellitus, and coronary heart disease. Emerging data suggest that FGF21 is a regulator of local and systemic metabolic homeostasis [23]. Based on the published role of FGF21 on fuel energy, oxidative stress, lifespan, its pleiotropic metabolic actions; and the fact that chronic metabolic states may increase FGF21 levels [24–28]; we decided to investigate serum FGF21 levels in breast cancer patients at diagnosis of the primary disease.

It has been well reported that serum contains a rich untapped source of disease-specific information. We were the first group to publish that FGF21 was a promising prognosis biomarker in ccRCC [29]. Since we reported that high serum FGF21 levels were associated with worse prognosis in terms of disease relapse in ccRCC patients; only a few clinical studies have reported the role of FGF21 in other cancer types [30–32].

However, up to date, very little is known regarding the role of circulating FGF21 as an oncologic biomarker, which could improve early diagnosis and define individual patient outcomes. In this current paper, we have studied the circulating FGF21 levels at diagnosis of the primary breast cancer. More interestingly, we have followed up on the clínical outcome of the breast cancer patients evaluated at diagnosis for over a decade to determine serum FGF21 usefulness as a prognosis biomarker.

Materials and methods

Population

Serum samples and associated individual data were obtained from the Serum Biobank "Biobanco de Muestras Públicas Oncológicas", from the Oncology Institute "A. H. Roffo", Buenos Aires Argentina. Patients included in this study were virgin of previous tumors and treatments. The institutional review boards (Instituto de Oncología "Ángel H. Roffo" (IOAHR) approved the protocol. The studies were done following the ethical principles outlined in the Declaration of Helsinki and compliance with the International Conference on Harmonization Good Clinical Practice guidelines. Every patient provided written informed consent before study-related procedures were done. This study included 45 breast cancer patients [Age; Median (range): 59,4 (32–88)]; [stage (S) distribution: 18 SI, 16 SII and 16 SIII; 4 non determined], and 51 age-matched healthy controls 25 [42 (23–63)]. The average breast cancer patient's follow-up period was 8,19 years (5 months-15.4 years).

Patients´ clinical, pathological features and metabolic parameters were obtained upon reading the corresponding hard copy charts. The parameters, which correlation with the serum FGF21 levels at diagnosis were analyzed, are depicted in Tables 1, 2 and 3. We evaluated metabolic parameters (cholesterol, triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), leptin and blood glucose levels (Table 1), clinical and pathological characteristics (age, tumor stage, histology sub-type, progesterone, estrogen and Her-2 neu receptors expression) (Table 2). The correlation between serum FGF21 levels and the body mass index (BMI) was analyzed as

Table 1 Association between serum FGF21 levels and breast cancer patients´ clinical and pathological parameters

Features		*N* of Patients with Positive FGF21 Levels/Total (%)	Test X^2 *p* Value
Age (*n* = 45)	< 60	9/23 (39.1)	*p* = 0.18
	≥ 60	13/22 (59.1)	
Tumor Stage (*n* = 42)	I	11/18 (61.1)	*p* = 0.79
	II	7/16 (43.8)	
	III	3/6 (50,0)	
	IV	1/2 (50,0)	
Nuclear Grade (n = 43)	**1**	**2/6 (33.3)**	*p* = 0.13
	2	**11/16 (68.8)**	
	3	**8/21 (38.1)**	
Histological Grade (n = 38)	**1**	**2/3 (66.7)**	*p* = 0.65
	2	**11/18 (61.1)**	
	3	**8/17 (47.1)**	
Tumor Size (n = 42)	**0**	**1/2 (50,0)**	*p* = 0,***57***
	I	**7/17 (41,2)**	
	II	**12/21 (57,1)**	
	III	**0/1 (0,0)**	
	IV	**1/1 (100,0)**	
Histology Sub-Type (n = 40)	**Ductal**	**18/32 (56.2)**	*p* = 0.11
	Lobular	**2/8 (25,0)**	
Estrogen Receptor (n = 45)	**Negative**	**3/6 (50,0)**	*p* = 0.95
	Positive	**19/39 (48.7)**	
Progesterone Receptor (n = 45)	**Negative**	**4/10 (40,0)**	*p* = 0.52
	Positive	**18/35 (51.4)**	
HER2 Expression (n = 45)	**Negative**	**20/38 (52.6)**	*p* = 0.24
	Positive	**2/7 (28.6)**	
Triple Negative (n = 45)	**No**	**20/43 (46.5)**	*p* = 0.14
	Yes	**2/2 (100,0)**	

No association was determined between each one of the clinical and pathological parameters analyzed and the circulating FGF21 levels in our cohort of breast cancer patients

Table 2 Correlation between the serum FGF21 levels and the metabolic parameters in breast cancer patients determined at diagnosis

Variable	FGF 21 Concentration	
	Spearman's test	*P* Value
Cholesterol	0.137	0.34
Triglycerides	**0.168**	**0.24**
Low Density Lipoprotein (LDL)	**0.101**	**0.48**
High Density Lipoprotein (HDL)	**0.024**	**0.87**
Leptin	**0.042**	**0.79**
Blood Glucose	**0.121**	**0.40**

The metabolic parameters analyzed showed no association with the FGF21 circulating levels in our cohort of breast cancer patients at the diagnosis of the primary disease

well (Table 3). The height (meters) and the weight (kg) of 38 of the patients included in our study were obtained from the charts, and their BMI was calculated as weight kg/height m^2. The evaluation and the consequent classification of patients based on their BMI result (weight kg/height m^2), as normal weight, overweight and having obesity, was performed according to the current guidelines for the treatment of obesity [33].

Table 3 Breast cancer patient´s distribution according to the BMI classification at the diagnosis of the primary disease

BMI (Kg/m2) Classification	*N*° of Patients/Total (%)	Md (range) serum FGF21 levels (pg/ml)	Serum FGF21 levels	
			Negative *N*° (%)	Positive *N*° (%)
Normal Weight	13/38 (34,21)	305,86 (28.41–725.40)	1 (7.7)	12 (92.3)
Overweight	10/38 (26,31)	124,20 (36.12-264.04)	6 (60)	4 (40)
Obesity	15/38 (39,47)	163,56 (40.52-479.85)	8 (53.3)	7 (46.6)

Pearson X^2 Test * $p = 0.015$

Serum samples

Serum samples were obtained from the serum biobank "Biobanco Público de Muestras Séricas Oncológicas" (BPMSO) of the IOAHR. According to the BPMSO standard procedure, 20 ml of blood were collected in tubes without any anticoagulant and left 15 min at 25 °C to allow the clot formation and centrifuged at 600 x g for 10 min. Then, serum was aliquoted and stored at -80 °C. Sera aliquots were used only once after thawing. Blood samples were drawn before surgery from untreated patients.

Immunoassays & Metabolism

Human serum levels of FGF21 were determined using R&D System® colorimetric ELISA test (Minneapolis, MN. Catalog Number: DF2100), following manufacturer's instructions. The samples variation intra- and inter-assay coefficients were 13% and 10% respectively. The minimum detectable dose (MDD) of FGF21 ranged from 1.61 to 8.69 pg/ml with a mean of 4.67 pg/ml. The ELISA was specific for human FGF21 with no cross-reactivity with human FGF19 and FGF23.

Serum Leptin levels were determined using R&D System® ELISA test (Catalog Number: DLP00) following manufacturer's instructions. The samples variation intra- and inter-assay coefficients were 3% and 8% respectively. The MDD of Leptin was lower than 7.8 pg/ml. FGF21 and Leptin concentrations were calculated from the standard calibration curve provided with the kits.

After fasting for 12 h, the subjects underwent the following laboratory blood analysis: glucose, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL) and triglycerides (TG). Glycaemia, LDL, HDL, triglycerides, and cholesterol values were determined by using the Cobas C311 Analyzer (Roche Diagnostics GmbH, Mannheim, Germany).

Statistical analysis

Listwise method was used to manage missing data. Initially we have determined that the expression of FGF21 doesn't have a normal distribution (Shapiro- Wilk Test; $p < 0{,}0001$ y Kolmogorov Smirnov Test; $p < 0{,}05$). Based on this result, we have decided to use statistic non- parametric tests in our statistical analysis. Differences in FGF21 levels among the groups were compared using the Kruskall-Wallis and Mann-Whitney tests, appropriate Md tests for even skewed data.

Sensitivity and specificity were calculated employing the optimal cut-off point. A ROC (Receiver Operating Characteristic) Curve [34] determined a cut-off point value of 130,64 pg/ml to define a sample as positive (or high) and negative (or low) for FGF21 serum concentration. Those samples that showed a serum FGF21 level ≥ 130,64 pg/ml were considered as positive. For statistical purposes, all variables were dichotomized into a low and a high FGF21s serum level groups. Dependence among the variables and the clinic-pathologic parameters was evaluated by $\chi 2$ test. The disease-free survival (DFS) was considered the length of time between the absence of disease determined by imaging and the reappearance of signs or symptoms. The overall survival (OS) was determined as the time length from diagnosis up to the patient death or last recorded information. Survival curves were plotted according to the Kaplan-Meier method and analyzed employing the Log Rank test. The Cox Proportional Hazards analysis was performed to evaluate whether the correlation between FGF21 serum levels and survival persist when other parameters were incorporated in the analysis. SPSSR 22 for Windows software package was used for statistical analysis [29].

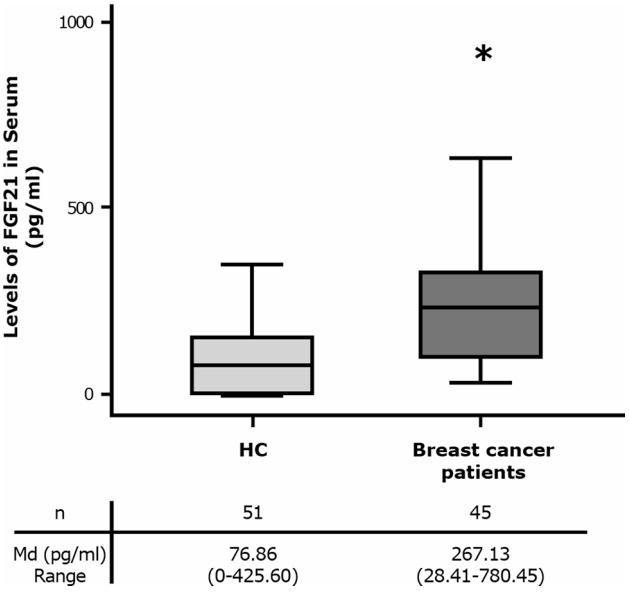

Fig. 1 Serum FGF21 levels in healthy controls (HC) and breast cancer patients. Serum FGF21 levels were significantly higher in breast cancer patients at diagnosis of the primary disease as compared to age-matched healthy controls (Kruskal Wallis test: $p < 0.0001$)

Results

Serum FGF21 levels in healthy controls

Previously to this work, we evaluated the circulating levels of FGF21 in human HC ($n = 51$) and its association with age and gender to rule out undesired variations [29]. No association was observed between FGF21 serum concentration and gender of HC (MW test) or age (Spearman's rank correlation test) [29]. In addition, HC population was dichotomized into those who had 60 years or more (≥ 60) and those who had less than 60 years (< 60) at the moment of the blood drawn. No statistical difference was observed between these groups (data not shown).

Serum FGF21 levels as diagnostic biomarker in breast cancer patients

In a previous work [29] we reported the expression of serum FGF21 in a healthy population. We demonstrated that serum FGF21 concentration is independent of gender or age variables. In addition, as FGF21 has a crucial role as a metabolic regulator, we showed that FGF21 levels are not correlated either with the concentration of leptin, triglycerides, LDL, HDL, or total cholesterol or with glycemia.

Here, we demonstrated that patients with breast cancer had significantly higher levels of serum FGF21 (median 267.13 pg/ml, range 28.41-780.45) as compared to the level determined in the Healthy Controls (76.86, 0.00-425.60) (KW and MW tests, $p < 0.0001$) (Fig. 1).

Using the ROC curve analysis, we determined that 130.64 pg/ml of serum FGF21 was the reference optimal cut-off value, close to the inflection point on the curve (data not shown), which maximized sensitivity and specificity. Employing this reference value, positive or high and negative or low serum FGF21 values were defined. In our population the sensitivity to diagnose breast cancer was 66.67% while the specificity was 64.71%. Based on this cut-off value, 30/45 (66.67%) breast cancer patients had positive serum FGF21 values as compared to 18/51 (35.3%) of the healthy controls (HC).

The quantification of serum FGF21 levels in untreated breast cancer patients ahead of surgery could be useful as a diagnostic biomarker.

Serum FGF21 and clinicopathological parameters in breast cancer

We studied whether serum FGF21 levels are modified by the stage or the histologic type of breast cancer. As shown in Fig. 2, no significant association was observed between the breast cancer stages (Fig. 2a) and serum FGF21 levels and no association was determined between histology

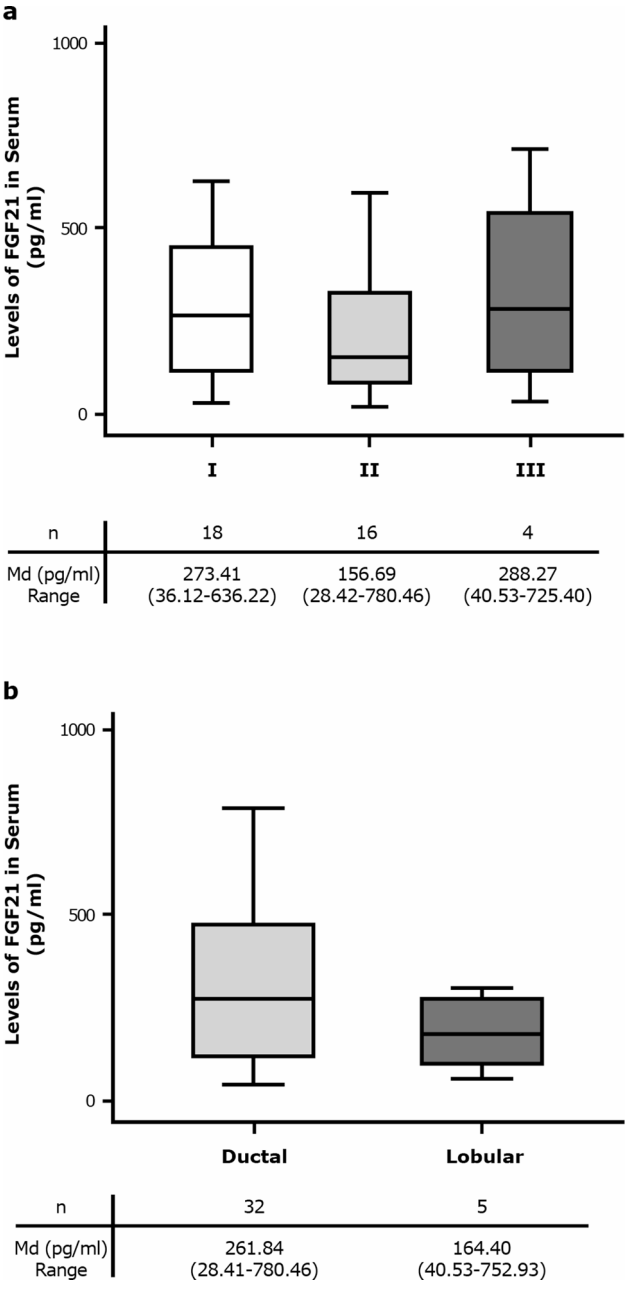

Fig. 2 Analysis of the circulating FGF21 levels in breast cancer and the tumor stage and histology. (**a**) No significant association was found between the breast cancer stages and serum FGF21 levels. (**b**) No association was determined between histology breast cancer sub-types and this growth factor circulating levels

breast cancer sub-type and this growth factor circulating levels (Fig. 2b).

In order to gain further insights into the clinical relevance of serum FGF21 levels in breast cancer, we analyzed possible associations between this growth factor and the main clinicopathologic features employed in breast cancer (Tumor size, metastatic lymph nodes, tumor stage, age, histological type, nuclear grade, hormonal receptors status, etc.). FGF21 values were dichotomized into "low" or "high" circulating levels using as cut-off point value 130.64 pg/ml corresponding to the 50th percentile of breast cancer patients' serum FGF21 concentration. This analysis was performed applying the Chi-Square Test. No association was observed between serum FGF21 levels and patients´ age, tumor stage, nuclear grade, histological grade, histology sub-type, estrogen and progesterone receptors expression and HER2 expression (Table 1).

Association between serum FGF21 levels with circulating metabolic parameters

Since FGF21 is a metabolic homeostasis regulator and a close link between obesity and breast cancer has been suggested, we investigated whether there was an association between serum FGF21 levels and the main parameters that account for a lipid profile.

In our cohort of breast cancer patients, no association among serum FGF21 levels and cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL), leptin, and blood glucose levels at diagnosis was determined. The Spearman`s correlation Rho Test was used for this analysis (NS: no significant) (Table 2).

Serum FGF21 levels and the body mass index (BMI)

Given the well-known connection between adiposity and breast cancer and the emerging functions of FGF21 as a metabolic and stress regulator; we analyzed the potential correlation between serum FGF21 levels and the breast cancer patients´ body mass index (BMI). Based on the BMI values, we have categorized the patients as having normal weight, overweight or obesity, according to the current guidelines for the treatment of obesity (Table 3) [33].

Table 3 shows the distribution of the breast cancer patients´ BMI evaluated according to the classification and the corresponding median (Md) and range concentration FGF21 levels in each one of the groups, also showing the number of positive (or high) and negative (or low) serum FGF21 levels determined at diagnosis of the primary disease in each one of the groups (Table 3).

The Pearson X^2 Test showed a statistically significant association between the BMI and the serum FGF21 levels determined at the diagnosis of primary breast cancer ($X^2 p = 0.015$). Surprisingly, the highest median serum FGF21 concentration corresponded to the group of patients with normal weight while both the obese and the overweight groups had lower values of circulating FGF21 compared to the normal weight group (Table 3).

Serum FGF21 levels and breast cancer patient prognosis

Serum FGF21 levels were evaluated in 45 breast cancer patients naïve of treatments at diagnosis of the primary disease. The ROC curve defined an optimal cut-off

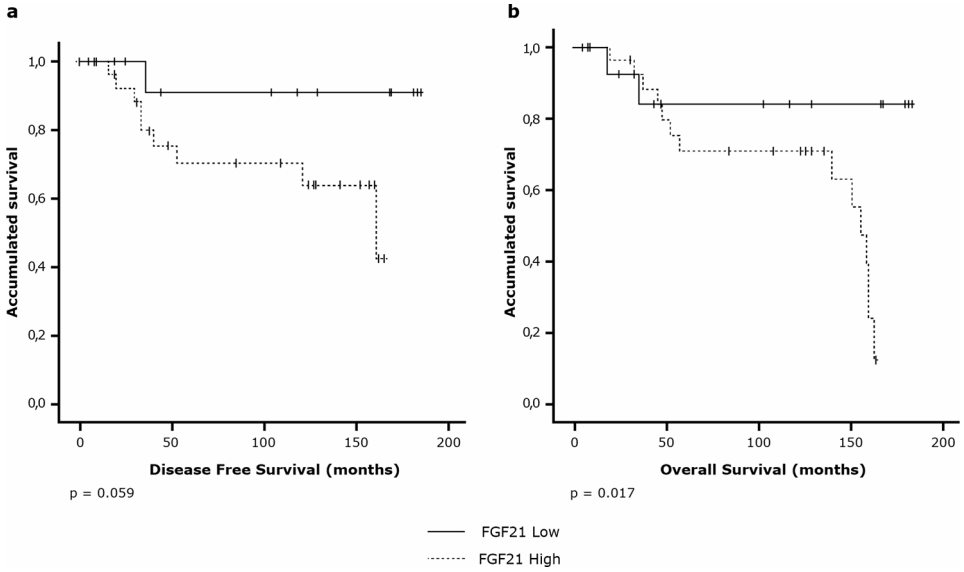

Fig. 3 FGF21 serum levels as a prognosis biomarker in breast cancer patients. (**a**) FGF21 serum levels at diagnosis were not associated with disease-free survival (Log Rank Test (Mantle Cox) $p=0.059$). (**b**) FGF21 serum levels at diagnosis correlated with overall survival. Patients with positive FGF21 levels experienced a poor prognosis showing a shorter OS (Log Rank Test [Mantle Cox] $p=0.017$)

point value of 130.64 pg/ml. The average of the follow-up period was Md 8.19 years (98.3 months) range (5 months-15.41 years).

The Kaplan-Meier method was used to estimate the disease-free survival (DFS) and the overall survival (OS). In univariate survival analysis, two-sided log-rank test for equality of survivor functions was used to assess the prognostic significance of different parameters on FGF21 positivity. Multivariate analysis was performed using the Cox proportional model to evaluate the predictive power of each variable independently of the others. We examined whether dichotomized variables of serum FGF21 expression could also predict disease-specific survival.

10/45 (22.2%) patients relapsed during the follow-up period. Among those patients who suffered a relapse 9/10 (90%) showed positive FGF21 serum levels at diagnosis [Median (Md) range 679.6 pg/ml (28.41-752.93 pg/ml)]. The Md (range) corresponding to the group of patients who did not relapse was 239.00 (36.12–725.4 pg/ml).

Serum FGF21 concentration at diagnosis seemed to be associated with poor DFS nevertheless it did not show statistical significance (Log Rank Test (Mantle Cox) $p=0.059$) (Fig. 3a). Serum FGF21 levels at diagnosis are associated with the worst prognosis in terms of OS. Breast cancer patients with negative FGF21 (< 130.64 pg/ml) circulating levels at diagnosis lived longer as compared to breast cancer patients with positive FGF21 serum levels (≥ 130.64 pg/ml) (Log Rank Test [Mantle Cox] $p=0.017$) (Fig. 3b). Furthermore, Cox-proportional hazards analysis demonstrated that the statistical significance between OS and FGF21 serum levels was maintained when variables such as histology type ($p=0.023$), tumor stage ($p=0.007$) or patient age ($p=0.033$) were included in the analysis.

Discussion

The role of FGF21 was initially elucidated in 2005 when it was proposed to be a novel metabolic regulator and a potential anti-diabetic drug [35]. FGF21 is expressed in several tissues such as liver [36], adipocytes [37], pancreas [38], brain [39], skeletal muscle, brown adipose tissue (BAT) and heart [40].

Since 2005 numerous articles have proposed that FGF21 is a powerful hormone that belongs to the list of factors that control energy homeostasis and metabolism [41–43]. It is secreted by the liver to regulate glucose metabolism in adipocytes in addition to insulin activity, and it serves as a potent regulator of lipid and energy metabolism [17]. Recent studies have indicated that FGF21 is a stress-responsive factor induced in response to metabolic or cellular insults [44].

Almost a decade ago, after studying the levels of FGF21 in the caloric restriction model of longevity, we hypothesized that the secretion of FGF21 by the liver in the bloodstream could act as an inter-organ messenger signal in metabolic alterations and even in cancer. In particular, we postulated that the increased FGF21 serum levels produced by the liver under stressful conditions may serve as an inter-organ feedback communication to minimize the stress-induced damage [29].

We were the first group in evaluating the potential value of FGF21 as a diagnostic and prognosis biomarker in cancer. We measured the FGF21 serum levels in clear cell renal cell carcinoma (ccRCC) patients before, and

now in a cohort of breast cancer. Initially, we reported that the median serum FGF21 level in our study was like previously reported values, and we published the results obtained in the group of ccRCC patients evaluated [29].

Now in this study we demonstrated that patients with breast cancer had significantly higher levels of serum FGF21 as compared to the level determined in the Healthy Controls (KW and MW tests, $p < 0.0001$). In our population, the sensitivity to diagnose breast cancer was 66.67% while the specificity was 64.71%. Applying a ROC Curve we determined a reference cut off value of 130 pg/ml. This defined positive (or high) and negative (or low) serum FGF21 levels in breast cancer patients at the time of the primary tumor diagnosis. Based on this cut off value, 30/45 (66.67%) breast cancer patients had positive serum FGF21 values as compared to 18/51 (35.3%) of the healthy controls. (HC). After observing the diagnostic value of FGF21 in breast cancer patients, we decided to conduct this extensive follow-up of the evaluated patients for over a decade to determine its usefulness as a prognosis biomarker. Circulating FGF21 showed prognosis usefulness when determined at the diagnosis of the primary breast cancer. Breast cancer patients with positive or high FGF21 serum levels at diagnosis had a shorter overall survival.

Interestingly, given the close link between FGF21 and metabolism regulation, it was rational to infer that FGF21 might be also related to changes in major metabolic markers.

Like in our ccRCC study, in our breast cancer patients cohort, no association was found between serum FGF21 levels and cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), leptin, and blood glucose levels at diagnosis. However, there was a statistically significant correlation between serum FGF21 levels, measured at the diagnosis of the primary breast tumor, and the body mass index (BMI) of the patients evaluated. Extensive research has demonstrated the increased risk for breast cancer, associated with obesity [45] and it has been reported that the BMI correlates with the degree of adiposity in the majority of the population [46]. In this current study, a correlation between serum FGF21 levels and the BMI of breast cancer patients was found ($X^2 p = 0.015$) and the distribution of positive and negative FGF21 levels among patients with normal, overweight and obesity has been categorized. Surprisingly, the group of breast cancer patients having normal weight, based on the BMI analysis, had the highest levels of circulating FGF21 at the diagnosis of the primary oncology disease. These results suggested that the significant higher serum FGF21 levels found at the diagnosis as compared to the healthy control individuals might not be determined necessarily by the extend of the adiposity. A very elegant work has shown that levels of functional FGF21 did not necessarily relate with obesity and metabolic features [47]. Despite that the initial studies have reported well an association between circulating FGF21 levels and overweight and obesity [38], more recently, other investigators have studied the relation of circulating FGF21, and BMI and the results were controversial [48, 49]. While analyzing these results, it is important to highlight that the main organ producing FGF21 is the liver. Taking this into account, the liver together with potentially the tumor cells could be the responsible for the significant higher serum FGF21 level in the breast cancer patients as compared to the healthy control group analyzed.

We were the first group to report that the circulating levels of FGF21 have a promising biomarker role in cancer progression beyond liver pathology. Following our first report on circulating FGF21 as a diagnostic and prognostic biomarker [29], different papers have corroborated our findings [48–53]. However, no studies including a decade follow-up survival period, as it is being shown herein, have been published. It is believed that FGF21 may act to reduce adiposity and subsequent estrogen accumulation which promotes the development of breast cancer [52].

A more recent study has shown that serum FGF21 levels can be utilized for monitoring the responses of breast cancer patients to treatment with tamoxifen and aromatase inhibitors. In that paper, treatment with tamoxifen and aromatase inhibitors for one year in breast cancer patients showed a significant reduction in FGF21 levels. It is believed that FGF21 can act as a mediator or marker of inflammation from adipose cells which is restricted after the use of medication [48]. However, the potential underlying mechanisms of FGF21 in breast cancer have not been elucidated.

Breast cancer therapy has had significant improvements in the past years, not only targeting the tumor cells but also the tumor microenvironment cells [4]. However, breast cancer incidence has been rising over the years pointing out that there is an imminent need for successful therapies.

To gain further insights into the clinical relevance of serum FGF21 levels in breast cancer, we analyzed possible associations between this growth factor and the main clinicopathological parameters used in breast cancer (TNM, age, histological type, nuclear grade, hormonal receptor status, etc.). Interestingly, we didn't find any significant association between the serum FGF21 levels and the clinical pathological variables in breast cancer.

Remarkably, the lack of positive association between the serum levels of FGF21 and all the clinical, pathological, and metabolic parameters reinforces the idea of using the circulating FGF21 as a promising diagnosis and prognosis biomarker in breast cancer patients.

Our results indicated that the level of serum FGF21 is useful as a prognosis biomarker in terms of overall survival. Patients with positive FGF21 serum levels at diagnosis had a significantly worse overall survival. Regarding the disease-free survival, the serum FGF21 concentration at diagnosis seemed to be associated with poor DFS nevertheless it did not show statistical significance. Moreover, FGF21 serum levels determined at diagnosis of primary breast cancer in naïve of treatment patients were useful as a sensitive diagnosis biomarker. This fact is highly relevant due to the lack of diagnostic biomarkers for this disease.

Up-to-date; the role of FGF21 as a prognostic biomarker is under investigation. Based on our results and a few studies published, it is highly possible that FGF21 could be a universal cancer biomarker rather than a specific diagnosis biomarker for breast cancer. Our results showed increased serum FGF21 levels since the early stages of the disease, but no differences were observed among stages. Clearly, further studies are needed to elucidate the mechanisms behind the increased serum FGF21 levels in cancer patients. We could hypothesize that the elevated FGF21 levels observed in these patients could be due to a high FGF21 secretion by the liver and also by the tumor cells or the stress caused by microenvironment metabolic disorders. Alternatively, the initiation of the tumor itself could be considered as a stressful condition that induces an increased FGF21 secretion by mainly the hepatocytes or/and adipocytes in less degree. We have performed experimental studies with mice samples. Preliminary studies have shown that serum FGF21 levels were increased consistently after days of tumor initiation compared with non-tumor control mice (data not shown). Similar results were observed using syngeneic 4T1 and E0771 breast cancer; and B16F10 melanoma models. More robust studies are needed to elucidate the mechanisms of FGF21 in tumorigenesis. Briefly, we could envision that by using genetically modified mice and the CRE-Lox technology, we would be able to determine the source of FGF21 at the initiation of tumorigenesis and the effect on survival in mouse models.

In summary, after this meticulously designed one-decade follow-up study, our results indicate that serum FGF21 could be a promising diagnosis and prognosis biomarker in breast cancer patients. This will certainly contribute to improving the clinical management of breast cancer patients.

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Author contributions
SMR performed experiments, clinical chart evaluation, statistical analysis, and wrote the manuscript; EA analyzed patient outcome; MN performed statistical analysis; LY and SM contributed to the manuscript preparation; MSDL, conceived and designed research, funded acquisition, analyzed data, and wrote the manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethical approval
This research was approved by the Ethics Committee on Research protocols of the Institute of Oncology "Ángel H. Roffo„, School of Medicine of the University of Buenos Aires (UBA).

Consent to participate
All patients included in the protocol signed an informed consent.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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