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High levels of autotaxin and lysophosphatidic acid predict poor outcome in treatment of resectable gastric carcinoma

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ABSTRACT

Background: Although early-stage gastric cancer is a candidate for curative surgical resection, the absence of specific early symptoms results in a late diagnosis and consequently most patients present advanced or metastatic disease. Identifying noveland tumor-specific biomarkers is needed to increase early detection and match patients to the appropriate treatment. The present study focused on the possible prognostic role of Ectonucleotide Pyrophosphatase/Phosphodiesterase 2 (*ENPP2*)/Autotaxin (ATX) and lysophosphatidic acid (LPA) in Gastro-Esophageal Adenocarcinoma (GEA). High levels of ATX/LPA are associated with several malignancies including gastrointestinal tumors.

Methods: Using a bioinformatics analysis, the incidence of *ENPP2* mutations together with its expression in the tumor tissues and the correlation between the presence of mutations and the survival rate were examined in databases of GEA patients. Furthermore, circulating levels of ATX and LPA were studied retrospectively and longitudinally both in patients receiving frontal surgery and in patients receiving preoperative chemotherapy.

Results: Overall findings suggested that although *ENPP2* mutations occur at low incidence, their presence was associated with a particular poor Overall Survival (OS). Furthermore, removal of the tumour by surgery resulted in a decrease in serum ATX and LPA levels within five days, regardless of any previous chemotherapy. Basal circulating ATX were associated with the aggressive diffuse GEA and could be considered of negative prognostic value, mainly in combination models with circulating Carcino-Embryonic Antigen (CEA).

Conclusions: Based on these observations, clinical trials with ATX-targeted drugs and standard chemotherapy regimens may benefit from selecting GEA patients based on their levels of ATX, LPA and CEA.

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1. Introduction

Gastric cancer (GC) represents the fifth highest prevalence and fourth highest lethality worldwide, according to the Global Cancer Statistics 2020 (GLOBOCAN 2020) [1]. Early-stage gastric cancer can be treated with both endoscopic and surgical procedures; however, sinceitsinitial symptoms are unspecific, gastric cancer is often diagnosed late [2].

Perioperative chemotherapy (CHT) with FLOT4 (5-Fluorouracil, Leucovorin, Oxaliplatin and Docetaxel) represents the gold standard for patients with locally advanced and resectable gastric and gastroesophageal junction adenocarcinoma (GEA), resulting in improvement of overall survival (OS) (5-year survival rates, 36 % vs 23 %) and pathological response [3].

Autotaxin (ATX) is an enzyme belonging to the nucleotide pyrophosphatase/phosphodiesterase family with its gene designation *ENPP2*. Secreted ATX acts as a lysophospholipase D (LPD), converting extracellular lysophosphatidylcholine (LPC) into lysophosphatidic acid (LPA) [4,5]. The ATX/LPA axis plays a role in many different physiological and pathological processes such as angiogenesis, embryonic development, inflammation, fibrosis and obesity [6–8]. Several studies have highlighted the link of these signal molecules with carcinogenesis and tumor progression through their crucial role in immune escape, tumor microenvironment, cancer stemness and drug resistance [9–11].

For instance, ATX is highly expressed in several types of cancer, such as glioblastoma [12], melanoma [13], liver cancer [14] and renal cancer [15]. Overexpression of ATX promotes the migration, invasion and proliferation of cancer cells [16]. Because of increased expression, ATX can act as a 'gatekeeper' to control LPA signaling via its LPA receptors. Furthermore, ATX and LPA appear not only to be useful biomarkers, but also a potential therapeutic target [17]. This is evidenced by the development of several small molecule ATX inhibitors developed in the last years [18].

In gastrointestinal (GI) tumors, such as cholangiocarcinoma, hepatocellular and pancreatic carcinoma, *ENPP2* expression is up-regulated compared to normal tissue, whereas in colorectal cancer *ENPP2* is down-regulated [18]. Given this difference in ATX/*ENPP2* expression in GI tumors, we investigated the role of *ENPP2*/ATX in GEA tumors. First, we determined the incidence of *ENPP2* potential mutations in a cohort of GEA patients using bioinformatics analyses. Second, we examined whether mutations in ATX/*ENPP2* was associated with prognosis. Third, we measured the circulating levels of ATX and LPA in patients with GEA undergoing either upfront surgery or pre-treated with CHT. In both patient populations, the predictive/prognostic role of ATX and LPA was assessed.

2. Methods

2.1. Bioinformatics analysis

We performed an analysis of the clinical-molecular features of *ENPP2* alterations, focusing on gastric and GEA tumors, by accessing a publicly available dataset. [https://www.cbioportal.org/study/summary?id=666b112d854f636a3861773f]. *ENPP2* mRNA expression was evaluated in a dataset of GEA patients from TGCA using the GEPIA (Gene Expression Profiling Interactive Analysis) tool [http:// gepia2. cancer-pku. cn/] [19]. For details see supplementary methods.

2.2. Serum samples collection and serum markers detection

Serum samples were obtained from 85 patients with resectable GEA, who underwent surgery at IRCCS "Saverio de Bellis" and donated their blood to the institutional Biobank in the past 7 years (2017 to 2024). Thirty-nine of these patients were treated with perioperative chemotherapy and 46 went directly to surgery. Twenty-nine of the 39 pre-treated patients followed a perioperative FLOT regimen. Serum at the time of surgery and when available 5 days after surgery were analyzed. For the 29 patients who were treated with FLOT regimen, sera before the start of preoperative CHT treatment, at the time of surgery and after postoperative CHT treatment were also analyzed. For details and serum proteins detection see supplementary methods.

2.3. Statistical analysis

Patients' characteristics are presented as Mean and Standard Deviation (M±SD) for continuous variables, and as frequency and percentage (%) for categorical variables. All details are described in supplementary methods.

3. Results

3.1. Bioinformatics analysis of the mutational status and gene expression of ENPP2 in GEA patients

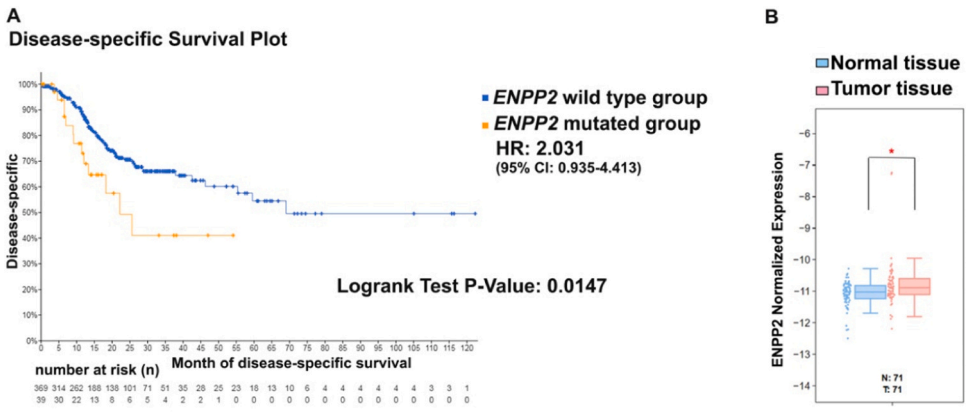
A total of 3055 GEA patients from fifteen studies contained in the current cBioPortal database of GEA studies were evaluated. *ENPP2* mutations were identified in 183 cases (6 %). The *TP53*, *TTN*, *MUC16* and *SYNE1* were the most frequent co-occurring mutations, with a frequency of 60.8 %, 48.0 %, 29.1 % and 23.3 %, respectively. Cancer-Specific Survival (CSS) was significantly shorter in patients with *ENPP2* mutations than in wild-type patients (22.19 vs 68.04 months, respectively; $p = 0.0147$, CI 95 %). The COX model was applied to calculate the risk of death (HR) based on the presence of mutations in *ENPP2*. Univariate analysis showed that the presence of mutations in this gene, substantially increased the risk of death compared to the wild type population, with a HR of 2.031 (95 % CI: 0.935–4.413) (Figure 1A). The observed mutations were all gain-of-function. This finding was corroborated by an additional bioinformatics analysis of *ENPP2* expression using the proteome GEPIA tool. The analysis of gene expression was assessed on tumor and healthy tissues. The data presented in Figure 1B show that there was little but significant ($p < 0.05$) over-expression of *ENPP2* in the tumor tissue compared to the adjacent normal tissue.

3.2. Selection of blood samples from two cohorts of GEA patients to assess ATX and LPA levels

We analyzed circulating levels of ATX and LPA in sera of patients from our institution who donated their blood to our Bio Bank. Circulating levels of ATX and LPA were measured before surgery (T pre-surgery) and 5 days after surgery (T post-surgery), both in the sera of directly resected patients and those pre-treated with CHT. In addition, a subset of patients receiving perioperative FLOT regimen was included in the pre-treated patient group. In this group of patients, the levels of the two biomarkers were measured in serum samples taken before preoperative CHT, before surgery and after postoperative CHT. The different group of patients are summarized in Figure 2. The differences between the two groups of patients, including chemotherapy administration, gender, age, histological type, clinical TNM (cTNM), are reported in Supplementary Table 1. For patients undergoing perioperative treatment, the degree of pathological remission, according to Beker's criteria [20], was assessed.

3.3. Surgery is associated with a decrease of circulating levels ATX and LPA in GEA patients

To investigate the effects of surgery, serum levels of ATX and LPA were measured by ELISA assay in blood samples obtained before (T pre-surgery) and after surgery (T post-surgery). The analysis was performed in patients who had immediate tumor resection and in patients who first were treated with preoperative chemotherapy. Significant reduction in ATX levels were observed in both patient groups regardless of any CHT



Summary:	Number of cases	Number of events	median month disease specific (CI 95%)
ENPP2 wild type group: 1813 pz	369	87	69.04 (55.43-NA)
ENPP2 mutated group: 183 pz (6%)	39	13	22.19 (13.35-NA)

Fig. 1. Bioinformatics analysis showing prognostic value of *ENPP2* alteration in GEA patients. A) Disease-specific survival graph demonstrates a significantly lower median survival (22.19 months) in the 183 patients with alterations in the *ENPP2* gene compared to the 1813 patients with wild type *ENPP2* (69.04 months), Log rank Test P-value = 0.0147. The mutation in *ENPP2* significantly increased the risk of death for patients carrying mutations in this gene (HR: 2.031 95 % CI: 0.935–4.413). The summary below showed the number of cases analyzed, the number of events occurred, and the median Cancer-Specific Survival (CSS) in both wild type and *ENPP2* mutated groups. B) *ENPP2* gene expression in tumor (red) and normal peri-tumor (blue) tissues from TGCA proteomic data analyzed by GEPIA tool, $p < 0.05$.

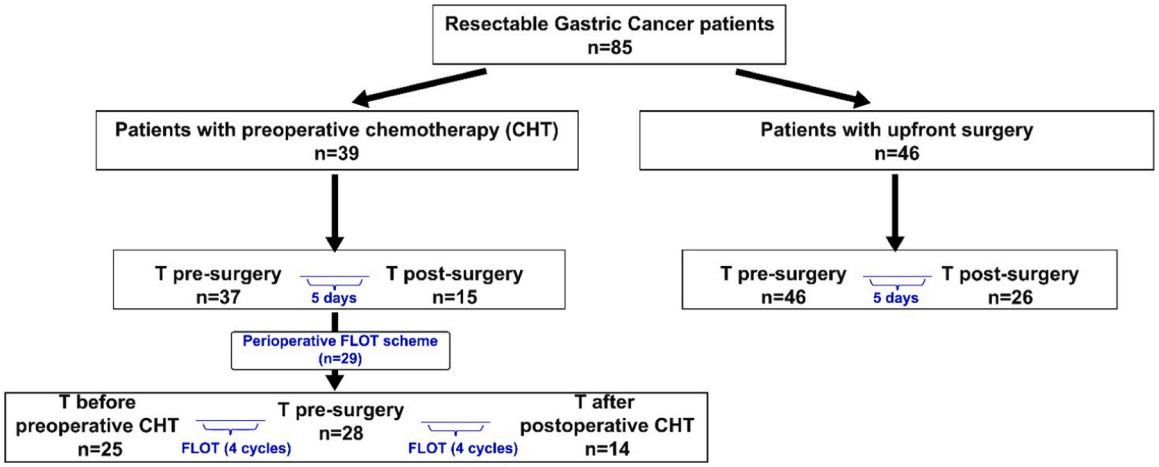


Fig. 2. Patient Groups. The sera from 85 GEA patients, of whom 39 were treated with preoperative chemotherapy prior to surgery and 46 received surgery only. In both cases, sera were obtained immediately before surgery (T pre-surgery) and 5 days after surgery (T post-surgery). In the setting of the pre-treated patients, we included a subgroup of 29 patients receiving perioperative FLOT regimen. In this group, sera were analyzed at time points before preoperative CHT, pre-surgery and after postoperative CHT.

pre-treatment, 246.13 ± 101.83 vs 198.08 ± 87.16 $p = 0.0005$ and 206.21 ± 104.47 vs 180.27 ± 72.73 $p = 0.003$, respectively. Similarly, significant decreases were detected in serum levels of LPA in patients without preoperative treatment with a 33.87 ± 10.51 μg/mL level at T pre-surgery and a reduction to 26.42 ± 10.9 μg/mL at T post-surgery ($p < 0.0001$). Among the patients with CHT pre-treatment, LPA levels decreased from 28.45 ± 10.76 μg/mL to 25.53 ± 12.43μg/mL ($p = 0.0001$) in the same time window. The graphs and the relative statistical analysis were reported in Figure 3 and Table 1.

At baseline, ATX and LPA levels were weakly correlated (Spearman's test:0.28, $p = 0.02$) for the entire cohort. In summary, it appears that the surgery was a determinant factor in reducing both ATX and LPA levels regardless whether patients received preoperative treatment or not.

3.4. Perioperative treatment with the FLOT regimen decreases circulating LPA and not ATX levels

In order to investigate the effects of chemotherapy treatment, we considered a subgroup of chemotherapy-treated patients who had received perioperative CHT with the FLOT regimen and whose sera were available before preoperative therapy, at the end of such therapy (T pre-surgery) and after the following postoperative therapy. ATX serum levels in the subgroup of pre-treated patients receiving FLOT remained almost unchanged when comparing values measured before preoperative CHT (186.21 ± 60.48 ng/mL), before surgery (190.99 ± 80.60 ng/mL) and after postoperative CHT (189.52 ± 48.23 ng/mL). By contrast, the reduction in LPA was significant ($p = 0.0002$) with values ranging from 29.63 ± 9.61 μg/mL measured before preoperative CHT to $23.61 + 10.90$ μg/mL ($p < 0.0001$) detected before surgery, and then

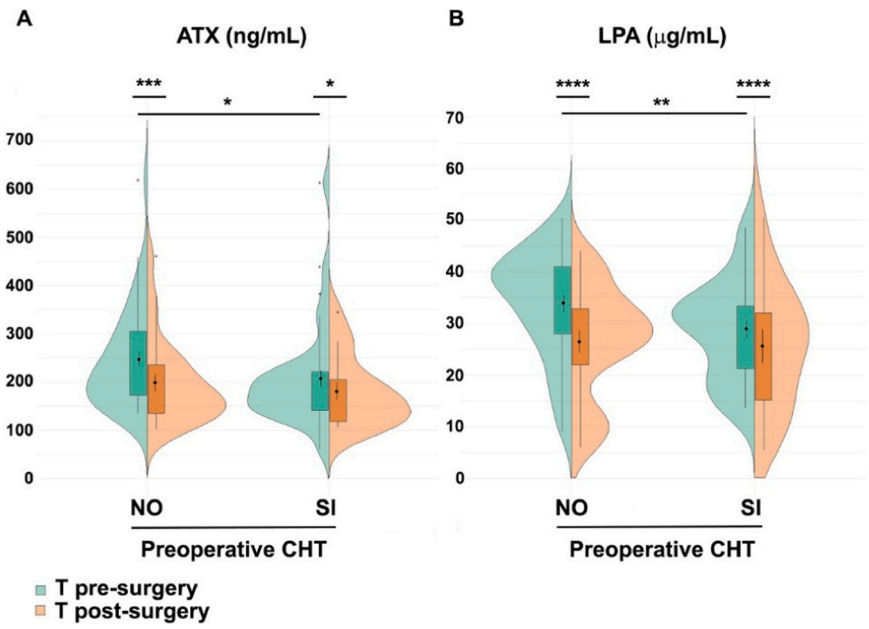


Fig. 3. Changes in circulating levels of ATX and LPA after surgery in GEA patients represented with half-violin plot. Serum levels of ATX (ng/mL) and LPA (μg/μL) were measured at the time before surgery (T pre-surgery) and 5 days after surgery (T post-surgery) in patients treated with preoperative CHT and in those receiving upfront surgery. The statistical analysis was reported in the Table 1.

Table 1
Changes in serum levels of ATX and LPA after surgery in GEA patients undergoing surgery, with or without prior CHT.

Parameters*	Preoperative CHT (No)($n = 46$)		p^	Preoperative CHT (Yes)($n = 39$)		p^	p†	pΨ
	T pre-surgery	T post-surgery		T pre-surgery	T post-surgery			
Autotaxin (ng/mL)	246.13 ± 101.83	198.08 ± 87.16	0.0005	206.21 ± 104.47	180.27 ± 72.73	0.03	0.02	0.49
LPA (μg/mL)	33.871 ± 10.511	26.423 ± 10.902	< 0.0001	28.445 ± 10.761	25.531 ± 12.429	0.0001	0.008	0.73

* As Mean and Standard Deviation for continuous variable.
^ Wilcoxon matched-pairs signed-rank test; † Wilcoxon rank-sum test (Mann–Whitney) among the parameters recorded at T pre-surgery; Ψ Wilcoxon rank-sum test (Mann–Whitney) among the parameters recorded at T post-surgery.
Abbreviations: LPA, Lysophosphatidic Acid.

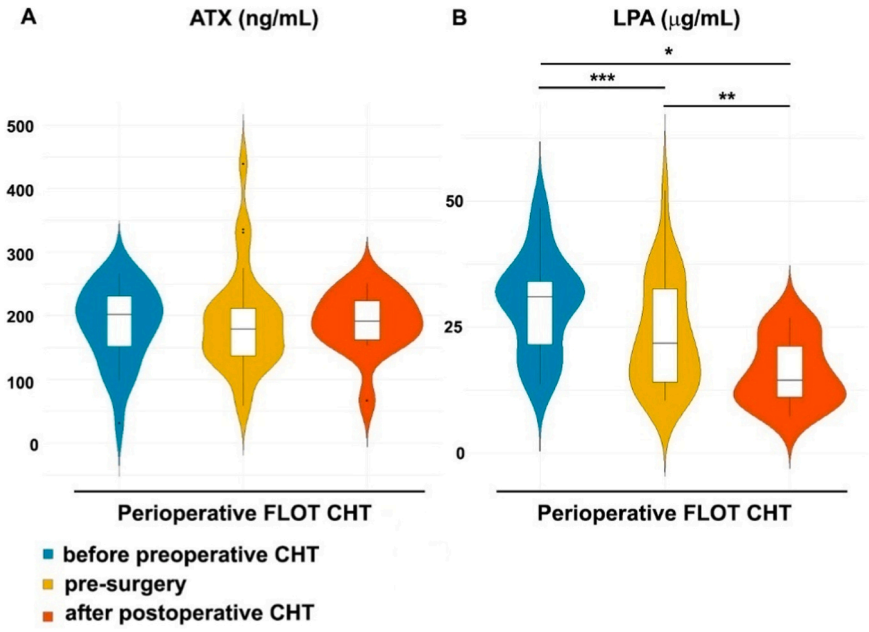


Fig. 4. Changes in circulating levels of ATX and LPA in GEA patients receiving FLOT perioperative treatment, represented with half-violin plot. Serum levels of both ATX (ng/mL) and LPA (μg/μL) were measured before preoperative CHT, before surgery and after postoperative CHT. Statistical analysis was reported in the Table 2.

decreasing further significantly (p = 0.02) to 16.11 ± 6.60 μg/mL at the end of postoperative CHT. The graphs and the relative statistical analysis are reported in Figure 4 and Table 2.

3.5. Gender and histological tumor type influence mean basal ATX serum values

Mean basal ATX serum valuesare influenced by sex of the patients. Females had a significantly higher value than males (274.38 +38 vs 190.56 +52.99 p = 0.0002). By contrast, no significant differences were found in the distribution of basal LPA values between the two genders (Table 3).

Baseline ATX and LPA levels were stratified by tumor site (Table 4). Higher basal LPA levels were significantly associated with antrum/pylorus (31.75 ± 10.78) and body/fundus (38.26 ± 59.81) disease compared to cardial (27.19 ± 14.17) and gastroesophageal (25.10 ± 73.52) sites. Similar although not statistically significant results were obtained for ATX levels.

Also, mean basal ATX value were different depending on the histological type using the Lauren classification [21]. A significantly higher ATX value was found in the diffuse subtype than in the intestinal and mixed type (250.67 +103.78 vs. 198.97 +77.55 vs.194.54 +60.00 p = 0.05) (Table 5).

3.6. Prognostic value of circulating ATX in GEA patients

ATX levels were associated with an increased risk in death (HR: 1.014) in subjects with ATX values greater than the median of 207.12 ng/mL. The models were also adjusted for covariates such as age, sex and histological grade (Table 6).

Using Kaplan-Meier OS calculation, ATX levels were associated with OS with a median follow-up of 12.12 months. Patient with median ATX levels ≤ 207.12 ng/mL had an improved OS than patients with ATX > 207.12 ng/mL (13.10 months vs 6.53 months, P = 0.09 CI 95 %) (Figure 5A). The negative prognostic value of ATX was improved by combining it with the serum tumor marker Carcinoembryonic Antigen (CEA) with cutoff of 5 ng/mL [22], whose basal levels were measured before any treatment. Interestingly, the median OS was significantly lower in patients with both markers increased (4.30 months for ATX > 207.13 and CEA > 5.0; log-rank test P-value: 0.009) (Figure 5B). The median OS was 15.87 months for ATX≤ 207.13 and CEA ≤ 5 vs 11.03 months for ATX > 207.13 and CEA ≤ 5 (for ATX ≤ 207.13 and CEA > 5 the calculation of the median OS was not applicable for the small number of patients in this category) (Figure 5).

4. Discussion

ATX/LPA axis is observed in a wide range of malignancies. There are limited studies examining the role of LPA/ATX in malignancies and its presence appears linked to resistance mechanisms, such as Epithelial-Mesenchymal Transition (EMT) and myofibroblast-associated immune suppression. In previous cell line studies, we observed that ATX/LPA axis influences the EMT of malignant cells. This effect was altered by the ATX inhibitor IOA-289 [18].

Previous studies suggested that the ATX/LPA axis in cancer can have a predictive/prognostic value when measuring circulating ATX/LPA levels [23]. Since GEA is a malignancy with a high EMT and myofibroblast content [24], we explored for the first time the role of *ENPP2*/ATX in GEA cancers. Bioinformatics analysis found *ENPP2* gene mutations in a large GEA patient population based on fifteen studies enriched for gastric cancer. While the mutations were low incidence

Table 3
Distribution of LPA and ATX at baseline among gender categories.

Parameters*	Gender		p^
	M (n = 51)	F (n = 34)	
Autotaxin(ng/mL)	190.56 ± 52.99	274.38 ± 115.48	0.0002
LPA(μg/mL)	30.690 ± 10.039	34.058 ± 10.872	0.17

* As Mean and Standard Deviation for continuous variables.
^ Wilcoxon rank-sum test (Mann–Whitney).
Abbreviations: LPA, Lysophosphatidic Acid; M, Males; F, Females.
The methods of definition of sex/gender were self-report.

Table 4
Distribution of Autotaxin and LPA at baseline among tumor site categories.

Parameters*	Tumor Site				p^
	Antrum/ pylorus (n = 45)	Body/ Fundus (n = 22)	Gastroesophageal (n = 9)	Cardias (n = 8)	
Autotaxin (ng/mL)	243.95 ± 109.37	211.03 ± 67.67	174.22 ± 42.86	197.14 ± 57.09	0.23
LPA (ng/ mL)	31.75 ± 10.78	38.26 ± 59.81	25.10 ± 73.52	27.19 ± 14.17	0.009

*As Mean and Standard Deviation for continuous variables.
^Kruskal-Wallis rank test.
Abbreviations: LPA, Lysophosphatidic Acid

Table 5
Baseline LPA and ATX levels differ among histological subtype of GEA.

Parameters*	Histological Subtype			p^
	Intestinal (n = 29)	Diffuse (n = 37)	Mucinous/Mixed/ Undefined (n = 19)	
Autotaxin(ng/ mL)	198.97 ± 77.55	250.67 ± 103.78	194.54 ± 60.00	0.05
LPA (μg /mL)	31.923 ± 10.774	33.006 ± 9.859	28.790 ± 11.935	0.47

* As Mean and Standard Deviation for continuous variables.
^Kruskal-Wallis rank test.
Abbreviations: LPA, Lysophosphatidic Acid

Table 6
Cox regression model on ATX and LPA inserted single in the model.

Parameters	HR	se (HR)	p-value	95 % (C.I.)
Autotaxin	1.014	0.005	0.006	1.004 to 1.024
LPA	1.000	0.001	0.942	0.999 to 1.000

^Abbreviations: HR, Hazard Ratio; se (HR), Standard Error of HR; 95 % (C.I.), Confidence Interval (C.I.) at 95 %; LPA, Lysophosphatidic Acid.
^Adjusted for Age, Gender, and Histological Grade.

Table 2
Variations of serum levels of ATX and LPA in GEA patients receiving FLOT perioperative CHT (*n = 29*).

Parameters *	Times (T)			p^	Multiple Comparisons		
	$T_{before\ preoperative\ CHT}$ *(a)*	$T_{pre\text{-}surgery}$ *(b)*	$T_{after\ postoperative\ CHT}$ *(c)*		*(b)* vs (a)	*(c)* vs *(a)*	*(c)* vs *(b)*
Autotaxin (ng/mL)	186.21 ± 60.48	190.99 ± 80.60	189.52 ± 48.23	0.85	0.31	0.99	0.99
LPA (μg/mL)	29.625 ± 9.614	23.612 ± 10.895	16.111 ± 6.597	0.0002	< 0.0001	0.002	0.02

The values were expressed as mean ± standard deviation. ^: Friedman test was used to test the variation over time of paired parameters as LPA and ATX; ¥: Wilcoxon matched-pairs test was used to calculate p-value between matched-pairs values. Abbreviations: LPA, Lysophosphatidic Acid.

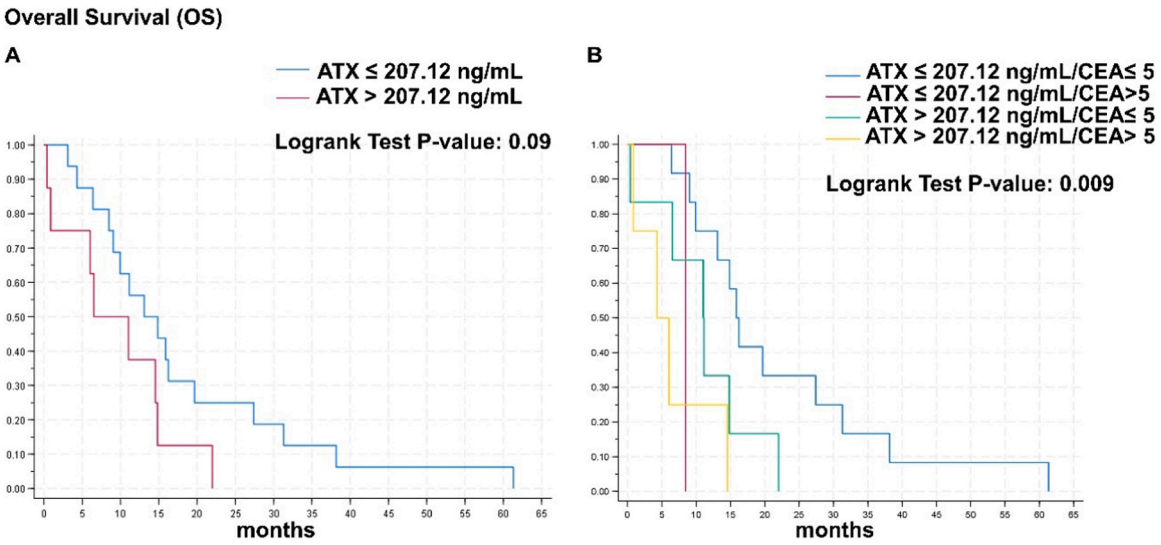


Fig. 5. Prognostic value of ATX and CEA in GEA patients. Overall survival (OS) plot using Kaplan-Meier method. A) ATX categories were calculated based on the median value (207.12 ng/mL) of the whole population at the basal level (Log rank Test P-value: 0.09); B) Survival curves derived from the four different combinations of ATX & CEA (Log rank Test P-value: 0.009).

(6 %), they were all gain-of-function likely to result in over-expression of the gene in the tumour compared to healthy tissue. Furthermore, the presence of mutations in *ENPP2* represented a significant risk factor of poor prognosis compared to patients with absence of mutations (HR: 2.031 CI: 0.935–4.413 95 %). Considering disease specific death events, median OS of patients with *ENPP2* mutation was significantly lower than patients with wild-type *ENPP2* (22.19 vs 69.04 months).

We postulated that reducing the tumour burden would be associated with a reduction in circulating ATX or LPA or both. Our retrospective study including a longitudinal analysis showed that removal of the tumour mass by surgery resulted in a decrease in serum ATX and LPA levels within five days, regardless of whether patients received chemotherapy.

We assessed the effect of chemotherapy on changes of circulating ATX and LPA in a subgroup of patients receiving perioperative FLOT regimen, consisting of four cycles of therapy before surgery and four cycles after surgery [3]. The effects of chemotherapy consisted of a progressive decrease in LPA, while ATX levels remained stable. It is possible that the ATX activity was reduced, which our ELISA assay did not capture. This may explain why the LPA levels decreased as result of the chemotherapy. However, ATX activity assays require a specialized set up, which may be considered for future studies [25].

Interestingly, we observed a correlation between basal LPA levels and the site of disease. Indeed, higher LPA levels were found in the antro-pyloric and body/fundus sites than in the cardias and gastro-esophageal sites. The tumor affecting the antrum/pylorus and body/fundus sites generally has a different aetiology (particularly due to Helicobacter pylori and Epstein Barr virus infections) and is mainly associated with a diffuse histology compared with tumors in cardias and gastroesophageal sites. This finding is in line with higher ATX levels associated with the diffuse GEA subtype, known for its invasiveness [26]. This was especially observed in patients who underwent upfront-surgery, where diffuse GEA was over-represented (as shown in Supplementary Table 1).

The role of ATX/LPA axis as a potential marker of poor outcome was corroborated by additional bioinformatic studies. In other malignancies, the potential prognostic value of ATX/LPA was observed [27]. For example, serum ATX levels were significantly elevated in breast cancer patients [23]. Mazzocca et al. [28] found that serum LPA levels were higher in patients with HCC than in healthy controls or in patients with liver cirrhosis [29]. Also, tumors with high tumor burden (e.g., ovarian cancer) or the occurrence of metastases were associated with higher levels of circulating ATX [30]. Finally, increased levels of LPA were associated with an increased risk of poor prognosis in HCC [18]. We found that ATX levels of $\geq$ 207.12 ng/mL at baseline were prognostic for worse OS in patients GEA. The prognostic value of ATX was increased (Log rank Test P-value: 0.03) when ATX and CEA levels were combined. This finding is consistent with comparable survival prediction models including tumor markers are being combined [31]. A similar approach was evaluated in pancreatic cancer, where the tumor marker CA19–9 was combined with ATX and LPA to provide an improved prognostic measure for patients [32].

5. Conclusion

In summary, we here describe two novel findings with implications for the field of biomarker research in GEA tumours: first, *ENPP2* gene mutation carry a poor OS; second, circulating levels of ATX and LPA have the potential to provide additional prognostic value to patients with GEA. Several ATX inhibitors and LPA receptor antagonists are being clinically evaluated, mainly in non-cancerous diseases such as idiopathic pulmonary fibrosis [33]. However, extensive preclinical studies indicate that such inhibitors may have a place for future clinical research in malignancies with high degree of fibrotic component, an immune-deserted and pro-angiogenic microenvironment [14,34–36]. Given this potential, such inhibitors may be useful combination partners for inhibitors of angiogenesis, chemotherapy induced immunogenic death, and controlling radiotherapy-induced fibrosis [35,36]. It is encouraging to see that the ATX inhibitor IOA-289 is being explored in combination with Gemcitabine/Nab-paclitaxel in patients with metastatic pancreatic cancer (ClinicalTrials.gov ID NCT05586516). Therefore, our approach may support ATX-targeting drugsto match standard regimen to the appropriately selected GEA patients.

Ethical approval

Approval of Ethics Committee Informed Consent “BIOBANCA”: Prot. no. 379/C.E. 16/09/2020.

Supplementary material

Supplementary Methods, Supplementary Table 1, Supplementary Tables 2–3.

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CRediT authorship contribution statement

Dolores Stabile: Software, Data curation. **Annalisa Ferro:** Investigation, Data curation. **Maria Notarnicola:** Validation, Data curation. **Angela D. Ricci:** Writing – review & editing, Visualization, Validation. **Vincenza Lorusso:** Data curation. **Valentina De Nunzio:** Investigation, Formal analysis. **Alessia Fantasia:** Software, Data curation. **Sergio Coletta:** Software, Data curation. **Claudio Lotesoriere:** Writing – review & editing, Visualization, Conceptualization. **Michael Lahn:** Writing – review & editing, Writing – original draft, Visualization, Conceptualization. **Rosalba D'Alessandro:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Formal analysis, Data curation, Conceptualization. **Gianluigi Giannelli:** Writing – review & editing, Visualization, Validation, Resources, Conceptualization. **Rossella Donghia:** Writing – original draft, Software, Formal analysis. **Natasha Renna:** Investigation. **Matteo Centonze:** Writing – review & editing, Formal analysis. **Giampiero De Leonardis:** Software, Investigation. **Annalisa Schirizzi:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Data curation, Conceptualization.

Declaration of Competing Interest

Dr Michael Lahn is Chief Medical Officer at iOnctura SA and holds stocks in the company; **Dr Gianluigi Giannelli** is an Editorial Member for Journal Experimental Clinical Cancer Research and was not involved in the editorial review or the decision to publish this article. **Dr Rosalba D'Alessandro** is Guest Editor for Cancers and was not involved in the editorial review or the decision to publish this article. **All other authors** declare no conflicts of interest.

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Appendix A

Sex and gender equity in research (Sager). The Terms sex/gender were used appropriately and are self-reported in Informed Consent.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ejca.2024.115066.

Data Availability

Data present in this study are available on request from the corresponding authors.

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