Bile extracellular vesicles hold protein biomarkers for the early diagnosis of cholangiocarcinoma in individuals with primary sclerosing cholangitis

A. LAPITZ[1,2], M. GRIMSRUD[3,4,5], PM. RODRIGUES[1,2,6], M. VESTERHUS[3,7,8], M. AZKARGORTA[2,9], K. GRZYB[10], H. REIMS[10], F. ELORTZA[2,9], L. IZQUIERDO-SANCHEZ[1,2], M. PERUGORRIA[1,2,11], L. BUJANDA[1,2,11,12], L.AABAKKEN[5], V.PAULSEN[5], TH. KARLSEN[3,4,5], **JM. BANALES**[1,2,6,13], **T. FOLSERAAS**[3,4,5]

[1]Biogipuzkoa Health Research Institute, San Sebastian, Spain; [2]CIBERehd, Madrid, Spain; [3]Norwegian PSC Research Center, Oslo, Norway; [4]University of Oslo, Oslo, Norway; [5]Oslo University Hospital Rikshospitalet, Oslo, Norway; [6]IKERBASQUE, Bilbao, Spain; [7]University of Bergen, Bergen, Norway; [8]Haraldsplass Deaconess Hospital, Bergen, Norway; [9]CIC bioGUNE, Derio, Spain; [10]Oslo University Hospital, Oslo, Norway; [11]University of the Basque Country, Leioa, Spain; [12]Hospital Universitario Donostia, San Sebastian, Spain; [13]University of Navarra, Pamplona, Spain.

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1 Introduction

- **Cholangiocarcinoma (CCA)** presents a significant threat to individuals with **primary sclerosing cholangitis (PSC)**, with a 20-year cumulative incidence of approximately 15%.
- Early diagnosis is challenging due to overlapping symptoms, and recommended MRI/MRCP surveillance every 6-12 months often proves suboptimal in detecting early-stage cancer.
- PSC-CCA patients face a grim prognosis, with a median overall survival of 5-12 months in unresectable cases, making CCA the primary cause of PSC-associated mortality.
- There is a critical **need for more accurate early detection methods**, allowing access to potentially curative options like tumor resection or liver transplantation.
- Extracellular vesicles (EVs), small membranous spheres found in biofluids, have recently emerged as a potential source of biomarkers.

2 Aim

Identify new accurate **protein biomarkers** in bile EVs to **diagnose CCA in patients with PSC**.

3 Method

- **Isolation of bile EVs** from patients with isolated PSC (PSC, n=52), PSC with CCA (PSC-CCA, n=14), and PSC without clinical evidences of malignancy at sampling who developed CCA overtime (PSC to CCA, n=8).
- EV characterization by transmission electron microscopy (**TEM**), **immunoblot** and nanoparticle tracking analysis (**NTA**).
- Proteomic analysis of EVs by **mass spectrometry (MS)**.
- Evaluation of the diagnostic efficacy of proteins by **receiver operating characteristic** (ROC) curves.

4 Results

Table 1. *Demographic and clinical features of the study cohort (analyzed by MS)*

		PSC n = 52	PSC to CCA n = 8	PSC-CCA n = 14
Sex (% males)		71	88	86
Mean age at sampling (years)		39.5	45.3	56.1
Liver-TX (%Yes)		50	25	36
Bile collection method	ERCP (%)	63	100	71
	PTC (%)	0	0	14
	TX (%)	37	0	7
	Liver resection (%)	0	0	7
Bacterial cholangitis at time of sampling	Yes (%)	2	13	0
	No (%)	98	88	100
Cirrhosis at time of sampling	Yes (%)	19	13	14
	No (%)	56	75	79
	NA (%)	25	13	7
AJCC staging	0 (%)	-	0	7
	I (%)	-	0	7
	II (%)	-	13	7
	III (%)	-	25	43
	IV (%)	-	38	14
	NA (%)	-	25	21
Biochemical parameters				
AST (% high)		71	12	21
ALT (% high)		62	10	15
ALP (% high)		81	13	27
Bilirubin (% high)		33	6	19
CEA (% high)		0	0	4
AFP (% high)		2	2	4
CA19-9 (% high)		4	4	17

Figure 1. *Characterization of isolated bile EVs*

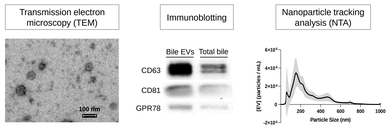
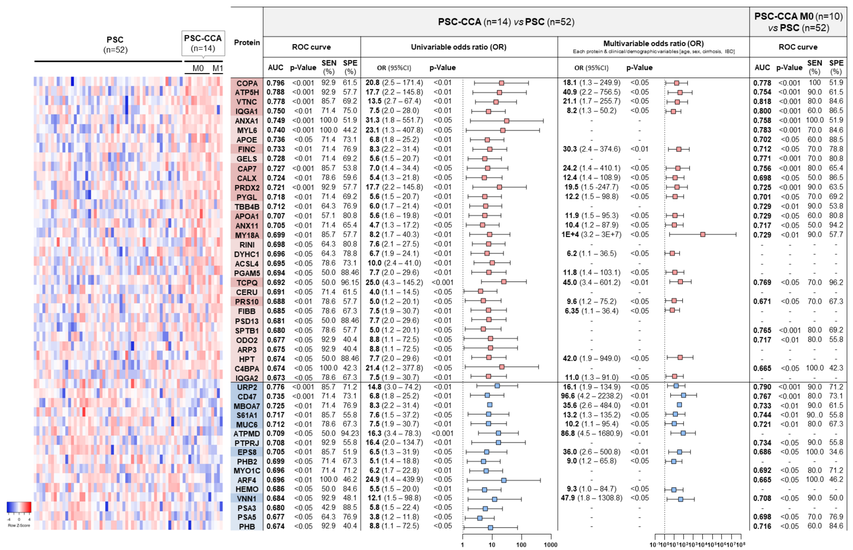
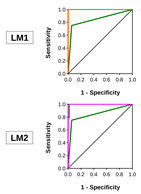


Figure 2. *Bile EV-protein biomarkers for CCA diagnosis in patients with PSC*



High-throughput proteomics of bile EVs identified 21 diagnostic biomarkers for PSC-CCA, regardless of sex, age, the presence of inflammatory bowel disease, or cirrhosis at the time of sampling. Among these, 14 biomarkers were observed to be more abundant (in red), and 7 exhibited lower levels (in blue) in patients with PSC-CCA compared to patients with isolated PSC. These 21 diagnostic biomarkers revealed a high diagnostic capacity for early CCA detection, as bile from patients with non-metastatic tumors had these biomarker levels altered.

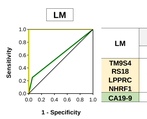
Figure 3. *Logistic models (LM) combining bile EV protein biomarkers for accurate CCA diagnosis*



LM	PSC-CCA (n=14) vs PSC (n=52)			
	AUC (95%CI)	p-Value	SEN (%)	SPE (%)
COPA ATP5H VTNC IQGA1 CALX PRDX2	**1.000** (1.000 – 1.000)	<0.0001	100.0	100.0
COPA ATP5H VTNC IQGA1 PRDX2	**0.996** (0.986 – 1.000)	<0.0001	100.0	98.1
CA19-9	**0.846** (0.692 – 1.000)	<0.001	75.0	94.3

Machine learning algorithms revealed COPA/ATP5H/VTNC/IQGA1/PRDX2 (AUC=0.996) and COPA/ATP5H/VTNC/IQGA1/CALX/PRDX2 (AUC=1.000) as highly effective in diagnosing PSC-CCA versus isolated PSC, surpassing the performance of CA19-9 alone (AUC=0.846).

Figure 4. *Logistic models combining bile EV proteins for the prediction of CCA development in patients with PSC*



LM	PSC to CCA (n=8) vs PSC (n=52)			
	AUC (95%CI)	p-Value	SEN (%)	SPE (%)
TM9S4 RS18 LPPRC NHRF1	**1.000** (1.000 – 1.000)	<0.0001	100.0	100.0
CA19-9	**0.596** (0.358 – 0.835)	ns	-	-

Machine learning algorithm TM9S4/RS18/LPPRC/NHRF1 demonstrated predictive capacity for CCA development in PSC before any clinical evidence of malignancy with 100% of sensitivity and specificity (AUC=1.000), whereas serum CA19-9 exhibited no significant predictive capacity for CCA development (AUC=0.596).

5 Conclusion

Bile EVs harbor valuable **protein biomarkers** for **predicting the development of CCA** and enabling **early diagnosis in individuals with PSC**.

Given the ease of bile collection during stenting for dominant strictures in individuals with PSC, this innovative liquid biopsy may be of significant value for monitoring disease progression and aiding access of potentially curative treatment options.

6 References

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7 Contact Information

ainhoa.lapitz@biodonostia.org
jesus.banales@biodonostia.org
trine.folseraas@medisin.uio.no

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