POSTER FPN 203P

An Innovative Evidence-Based Laboratory Medicine (EBLM) Test to Help Doctors in Multi-Cancer Early Detection (MCED)

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BACKGROUND

Introduction

- According to the International Agency for Research on Cancer (IARC), **cancer is among the leading causes of death worldwide**[1]. In 2022, there were almost 20 M new cases and 9.7 M cancer-related deaths worldwide. By 2040, new cancer cases per year are expected to rise to 29.9 M and the number of cancer-related deaths to 15.3 M[1]. **Approximately 40.5% of men and women will be diagnosed with cancer at some point during their lifetimes**[1].
- **There are 2 B people worldwide with overweight or obesity —including 124 M children—**, and these figures are expected to continue to increase[2].
- **Obesity is a risk factor for several major cancers**, including post-menopausal breast, colorectal, endometrial, kidney, esophageal, pancreatic, liver, and gallbladder cancer[3]. Excess body fat increases the risk of cancer mortality approximately by 17%[3].
- **Diet, obesity and lack of physical activity are listed as the main causes of cancer deaths attributed to non-genetic factors**[4]. **The link between obesity and cancer risk is clear**[4].

Key Facts about Cancer in the U.S.

- U.S. cancer endemic: the second leading cause of death[5], and growing.
- In 2024, an estimated 2,001,140 new cases of cancer will be diagnosed in the United States and 611,720 people will die from the disease[5].
- Most common cancers are breast, prostate, lung and bronchus, colon and rectum, melanoma of the skin, bladder, kidney and renal pelvis, non-Hodgkin lymphoma, endometrial, pancreatic, leukemia, thyroid, and liver cancer[5].

Most Studied Known or Suspected Cancer Risk Factors[6]

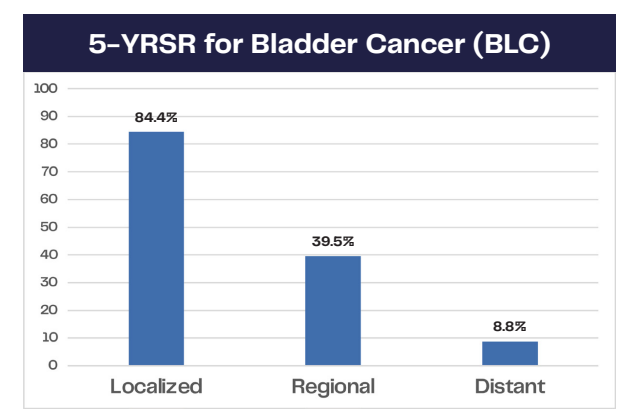
- Age
- Alcohol
- Cancer-causing substances
- Chronic inflammation
- Diet
- Hormones
- Immunosuppression
- Infectious agents
- Overweight and obesity
- Radiation
- Sunlight
- Tobacco

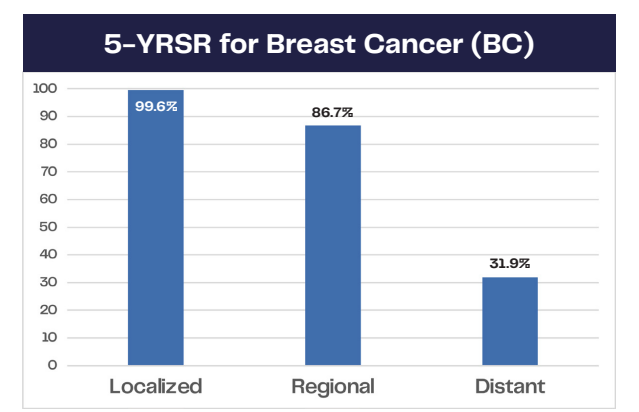
Cancer Prognosis and 5-Year Relative Survival Rate (5-YRSR)[7]

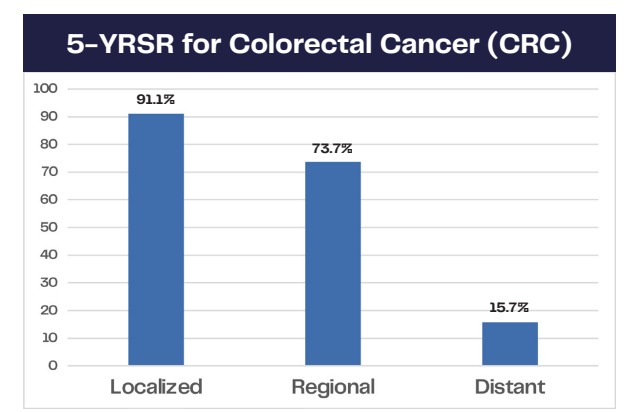
For all types of cancer, **the 5-YRSR is strongly associated with tumor stage at detection**.

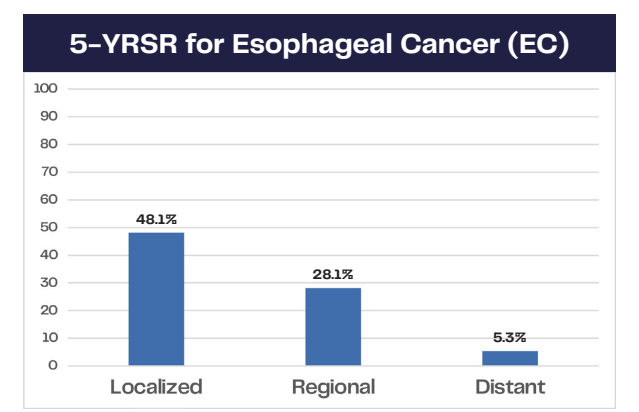
In this way, the 5-YRSR for localized cancers —detected early, when cancer is most likely to be treated with success— is the highest —up to the 99.9% for papillary thyroid carcinoma (PTC)—. However, **when cancer has metastasized to other parts of the body, the 5-YRSR drops** —to below 3.1% for metastasic lung cancer (LC)—. Apart from the stage of the cancer at the time of detection, many other factors such as the patient's age and health can also significantly affect the 5-YRSR.

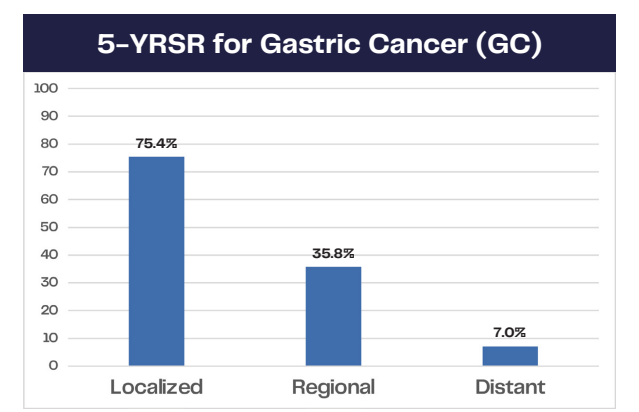
The 5-YRSR for some of the most frequent solid tumors, according to their stage at diagnosis —localized, regional and distant—, are[7] (alphabetically sorted):

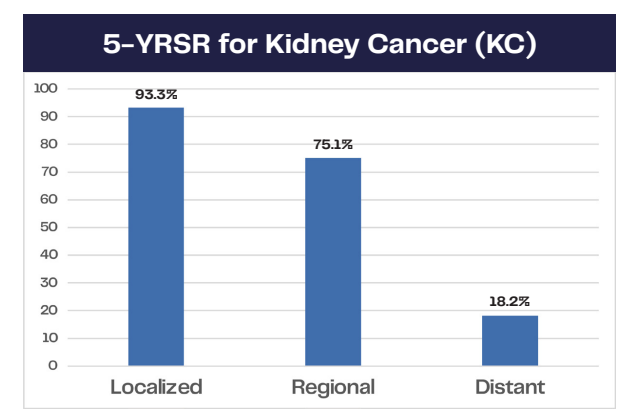


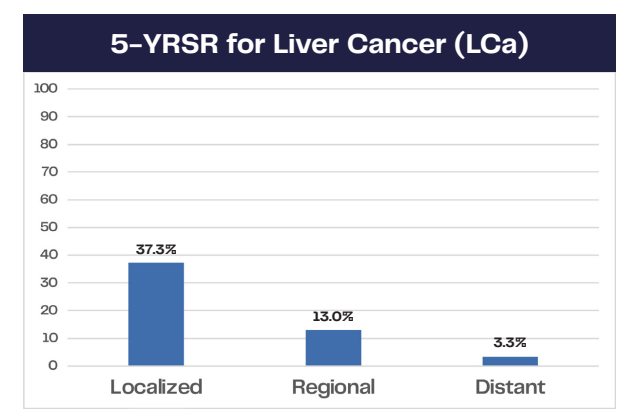


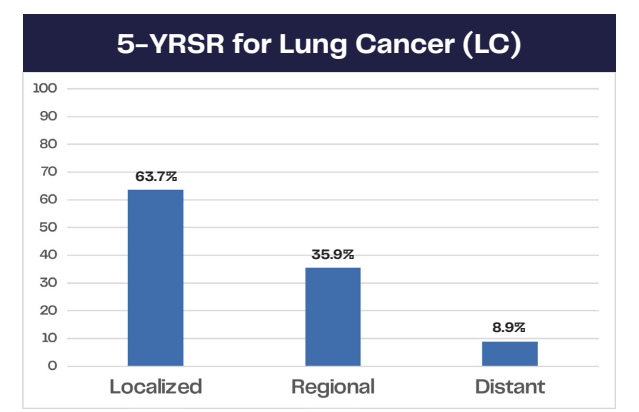


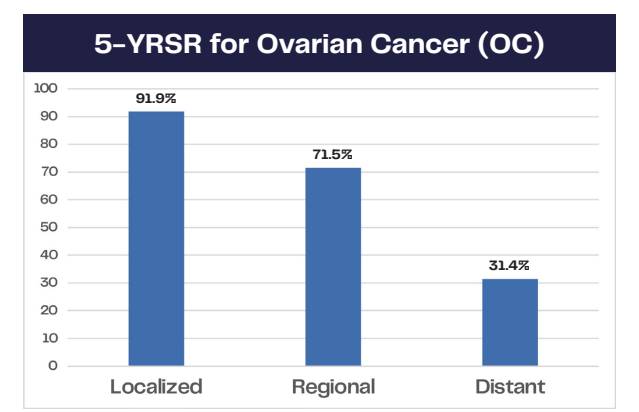


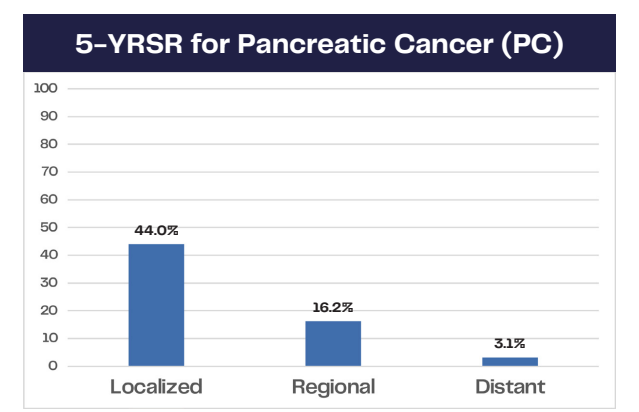


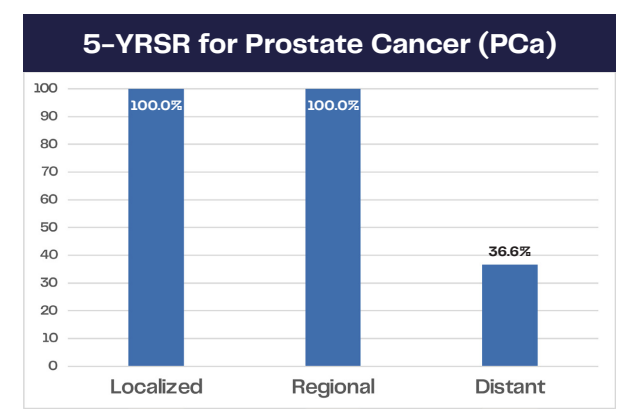


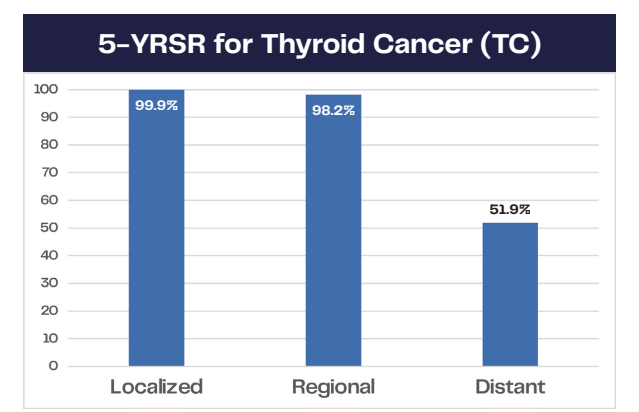












Current Tools to Diagnose Cancer

They are often invasive, expensive and may give many false positives (FP):

- TM laboratory tests (blood, urine and other fluid samples)[8,9,10,11]: cheap and fast, but inaccurate, as **other comorbidities, apart from cancer, can also increase serum TM levels**, thus **giving FP and reducing the specificity**.
- X-Ray: **very low accuracy** —high rate of FP due to scars, cysts or benign nodules—, and **radiation exposure**.
- Algorithms developed by the American College of Radiology (ACR)[12] for imaging procedures: BI-RADS for mammography, ultrasound (US) or magnetic resonance imaging (MRI); C-RADS™ for computerized tomography (CT) scan; GI-RADS for transvaginal (TV) US; LI-RADS® for US or CT-scan; Lung-RADS for CT-scan; O-RADS™ for US; PI-RADS® for MRI; or TI-RADS™ for ultrasonography. They are usually **expensive** and they have a **high FP rate**, which may lead to unnecessary invasive biopsies.
- Liquid biopsy: blood test that looks for circulating tumor DNA (ctDNA). Its **accuracy can vary depending on the type and stage of cancer, detecting low levels of ctDNA in early-stage cancers**. **Very expensive**.
- Tissue biopsy: **current gold standard in cancer diagnostics**, offering a detailed view of the tumor's cellular makeup. However, they are **highly invasive** and **expensive**.

Upcoming Non-Invasive Cancer Diagnosis Solutions

In recent years, tumor markers (TM) have been suggested to help in cancer recurrence surveillance, confirmation diagnosis and screening.

However, **TM can be also increased due to non-malignant diseases[9,11] (Table 1), mainly in the liver and kidneys, since TM are mainly catabolized in the liver —detoxification pathway—, and excreted through the kidneys**.

Therefore, any disease that affects these two organs will affect TM serum levels[9,11]. In this way, the **Spanish Society of Laboratory Medicine (SEQC^ML)** developed in 1994 a set of rules known as the **Barcelona Criteria**[9] to minimize FP:

1. Evaluate **serum TM concentration**.
2. Exclude the main source of FP results by **discarding benign pathologies (Table 1)**.
3. When moderate results, **perform follow-up —serial testing—**.
4. Eliminate high results by **avoiding technical interferences**.

TM/Comor.	Asc.	BPH	Cho.	CLD	Mod. Jau.	Acu. Jau.	Met.	Pan.	Pem./Pso.	Per./Ple. Eff.	Pro.	CKD
AFP	Moderate	Moderate		High	High	High			Moderate	Moderate	Moderate	Moderate
CA 15.3	High	High	High	High	High	High		High	High	High	High	High
CA 19.9	Moderate	Moderate	Very High	Moderate	Very High	Very High		Very High	Moderate	Moderate	Moderate	Moderate
CA 72.4	High	High	High	Very High	High	High		High	High	High	High	Very High
CA 125	Very High	High		High	High	Very High	High		High	Very High	High	High
CEA	Moderate	Moderate		High	High	High			Moderate	Moderate	Moderate	Very High
CYFRA 21-1	Moderate	Moderate		High	High	High			Moderate	Moderate	Moderate	High
HE4	Moderate	Mild		High	High	High			Mild	Mild	Mild	N/A
NSE	Moderate	Moderate	Moderate	Moderate	Moderate	Moderate		Moderate	Moderate	Moderate	Moderate	High
ProGRP	High	High	High	High	High	High		High	High	High	High	Very High
PSA	Moderate	Very High	Moderate	Moderate	Moderate	Moderate		Moderate	Moderate	Moderate	Very High	Moderate
SCC	Moderate	High	Moderate	Moderate	Moderate	Moderate		Moderate	Very High	N/A	High	N/A
S100	Mild	Mild	Mild	Mild	Mild	Mild		Mild	Mild	Mild	Mild	N/A

Table 1. Main common non-malignant causes of high serum levels of the major TM that are well-known sources of the most frequent false positives (FP). TM: tumor marker; Comor.: comorbidities; Asc.: ascites; BPH: benign prostate hyperplasia; Cho.: cholestasis; CLD: chronic liver disease; Mod. Jau.: moderate jaundice; Acu. Jau.: acute jaundice; Met.: metrorrhagia; Pan.: pancreatitis; Pem./Pso.; pemphigus/psoriasis; Per./Ple. Eff.: pericardial/pleural effusions; Pro.: prostatitis; CKD: chronic kidney disease. Mild: up to 2 times upper normality level (UNL). Moderate: between 2 and 5 times UNL. High: between 5 and 10 times UNL. Very High: more than 10 times UNL. N/A: extremely high increase in TM —even higher to levels found in cancer patients—, invalidate the use of these TM for these patients.

Case Study

A 51 y.o. woman got tested and the algorithm suggested that a cancer was present with a 97% probability, but without establishing the site. Nonetheless, it suggested that it could be a breast or a digestive cancer. Besides, the algorithm also suggested hepatic disease, specifically due to alcohol abuse —even though the patient stated "weekend social" in the alcohol intake question of the lifestyle habits section of the test requisition form (TRF), shown in **Figure 3**—. In this regard, given this discrepancy and after rechecking the patient about her alcohol intake, she acknowledged drinking more than just "weekend social".

Then, she decided to go ahead of the medical appointment planned for a week later —as part of the protocol defined in the randomized controlled trial (RCT)—, and went to her assigned general practitioner (GP) in the Spanish National Health System (SNHS)[13]. The response from her doctor was: "These types of analysis based on machine learning (ML) algorithms are not reliable at all and do not need to be further discussed, since you have neither symptoms nor signs".

As she was not convinced with this answer, she was referred to one of our collaborating private medical centers who prescribed her a mammography. Results showed that she had a mass in her right breast, so a CT-scan was performed afterwards, with a BI-RADS 5* result —according to the American Cancer Society (ACR)[12], with category 5 findings meaning that it had a 95% chance or higher of being cancerous—. The subsequent biopsy confirmed that she had invasive ductal carcinoma (IDC) Nottingham grade 2 breast cancer —the tumor was 2 cm or less and had not metastasized to the axillary lymph nodes—. At this point, early detection played its role and was able to find cancer in an early stage, resulting in a reduced lead time[14] (**Figure 1**).

The patient returned to her GP to request medical leave —in Spain, the SNHS is responsible for processing the statutory sick pay (SSP)—, and underwent surgery at the SNHS.

The RCT management wishes to state that the SNHS is undoubtedly one of the best health systems in the world. This case study is only intended to shed light on the potential of new cancer diagnostic tools based on ML algorithms as a tool to help healthcare professionals to improve diagnosis accuracy, reduce medical errors and misdiagnoses.

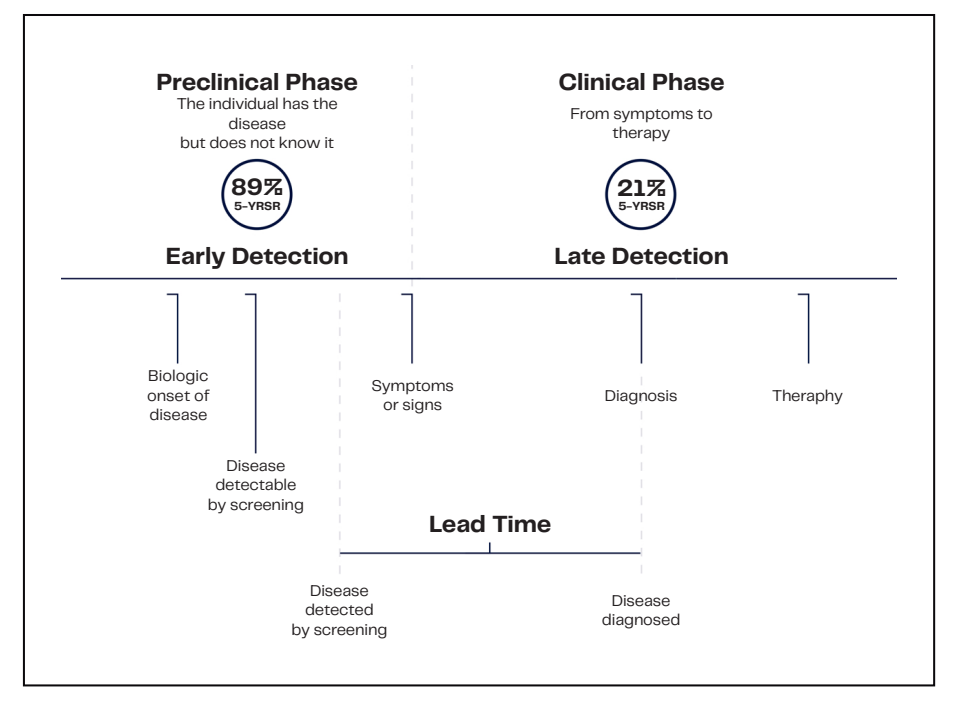


Figure 1. Lead time —the duration of time between the detection of the disease via screening and its diagnosis— plays a critical role in cancer detection. The shorter the lead time, the earlier the detection of the disease, the faster the next steps can be defined, potentially the better the prognosis and the lower treatment-related costs. The 5-YRSR decreases considerably if cancer is detected late —from 89% to 21%—. This higlights the need of early detection, as it saves lives.

TM/Comor.	Asc.	BPH	Col.	Cho.	CLD	Mod. Jau.	Acu. Jau.	Pan.	Pem./Pso.	Per./Ple. Eff.	Pro.	CKD	KF	UTI	DM	Sar.	CD	HypoT.	HyperT.	End.	OGBC	Preg.	Lip.	Hem.	Syn.	ALD	Hep.	Cirr.	PBC	HBD	AD	CS	HyperPTH	IBD	CD	HPAFP	ND	RA	PS	TBC	OPA	CVD	CAD	OIGD	ASCI	CLE	Ane.	OAD	Med.
ALAT							Moderate (↑)												Moderate (↑)					Moderate (↑)			Moderate (↑)	Mild (↓)																					
Albumin												Moderate (↑)	Moderate (↑)					Mild (↓)	Mild (↓)					Moderate (↑)				Mild (↓)						Mild (↓)															
ALP				Moderate (↑)		Moderate (↑)	Moderate (↑)					Moderate (↑)	Moderate (↑)		Moderate (↑)	Moderate (↑)	Moderate (↑)		Moderate (↑)			Moderate (↑)		Moderate (↑)			Moderate (↑)	Moderate (↑)	Moderate (↑)				Moderate (↑)							Moderate (↑)				Moderate (↑)					Moderate (↑)
Amylase			Moderate (↑)					Moderate (↑)				Moderate (↑)	Moderate (↑)											Mild (↓)																				Moderate (↑)					
ASAT							Moderate (↑)	Moderate (↑)																Moderate (↑)			Moderate (↑)	Mild (↓)																Moderate (↑)					
Calcium								Mild (↓)					Mild (↓)			Moderate (↑)		Moderate (↑)	Moderate (↑)					Moderate (↑)									Moderate (↑)																
Creatinine						Mild (↓)						Moderate (↑)	Moderate (↑)															Moderate (↑)																					
GGT				Moderate (↑)		Moderate (↑)	Moderate (↑)	Moderate (↑)				Moderate (↑)	Moderate (↑)		Moderate (↑)														Moderate (↑)												Moderate (↑)			Moderate (↑)					Moderate (↑)
Glucose															Moderate (↑)																																		
LDH				Moderate (↑)								Moderate (↑)	Moderate (↑)			Moderate (↑)							Mild (↓)	Moderate (↑)			Moderate (↑)																	Moderate (↑)					
Lipase						Mild (↓)		Moderate (↑)				Moderate (↑)	Moderate (↑)																																				
Potassium												Moderate (↑)	Moderate (↑)										Mild (↓)	Moderate (↑)							Moderate (↑)	Mild (↓)												Mild (↓)					Moderate (↑)
Sodium												Mild (↓)	Moderate (↑)		Moderate (↑)			Mild (↓)					Mild (↓)					Mild (↓)			Mild (↓)	Moderate (↑)																	
Total Bilirubin				Moderate (↑)		Moderate (↑)	Moderate (↑)																Moderate (↑)	Mild (↓)	Moderate (↑)		Moderate (↑)	Moderate (↑)																					
Total Cholesterol				Moderate (↑)		Mild (↓)	Mild (↓)											Moderate (↑)	Mild (↓)										Moderate (↑)																				
Triglycerides						Mild (↓)	Mild (↓)					Mild (↑)	Moderate (↑)		Moderate (↑)			Moderate (↑)									Moderate (↑)	Moderate (↑)																					
Urea												Mild (↑)	Moderate (↑)																																				
Vitamin D													Mild (↓)				Mild (↓)					Mild (↓)																											
AFP	Moderate (↑)	Moderate (↑)			High (↑)	High (↑)	High (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)									Very High (↑)					High (↑)	High (↑)		Very High (↑)						Very High (↑)	High (↑)			High (↑)	High (↑)							High (↑)	High (↑)
beta-HCG												Very High (↑)	Very High (↑)								High (↑)	Very High (↑)								High (↑)																		Very High (↑)	
Calprotectin																																		Very High (↑)	Moderate (↑)			Moderate (↑)											
CA 15.3	High (↑)	High (↑)			High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	High (↑)			Moderate (↑)			High (↑)		High (↑)						Moderate (↑)	Moderate (↑)		High (↑)									Very High (↑)	Moderate (↑)				High (↑)				High (↑)	
CA 19.9	Moderate (↑)	Moderate (↑)		Very High (↑)	Moderate (↑)	Very High (↑)	Very High (↑)	Very High (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)		Moderate (↑)						Very High (↑)									Very High (↑)									Very High (↑)	Very High (↑)	Moderate (↑)			High (↑)				High (↑)	
CA 72.4	High (↑)	High (↑)			Very High (↑)	High (↑)	High (↑)	80 (to 3x LSR)	High (↑)	High (↑)	High (↑)	High (↑)	Very High (↑)							High (↑)	Moderate (↑)									High (↑)									High (↑)					High (↑)					Very High (↑)
CA 125	Very High (↑)	High (↑)			High (↑)	High (↑)	Very High (↑)	High (↑)	High (↑)	Very High (↑)	High (↑)	High (↑)	High (↑)					High (↑)		Very High (↑)	High (↑)	High (↑)					Moderate (↑)	Moderate (↑)		High (↑)									Very High (↑)	Very High (↑)	Moderate (↑)	Very High (↑)		High (↑)				Very High (↑)	High (↑)
CEA	Moderate (↑)	Moderate (↑)			High (↑)	High (↑)	High (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Very High (↑)	Very High (↑)					High (↑)		High (↑)	Moderate (↑)							Moderate (↑)		High (↑)				Moderate (↑)					Very High (↑)	High (↑)				High (↑)				High (↑)	
CYFRA 21-1	Moderate (↑)	Moderate (↑)			High (↑)	High (↑)	High (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	High (↑)	Very High (↑)			Moderate (↑)														High (↑)									Very High (↑)	High (↑)	High (↑)								High (↑)
Cyr61		Moderate (↑)																																	Moderate (↑)			Moderate (↑)					Moderate (↑)						
PIVKA-II				Moderate (↑)		Moderate (↑)	Moderate (↑)																			Moderate (↑)																							Moderate (↑)
EGFR																																																	
Ferritin					Moderate (↑)							Moderate (↑)	High (↑)																																		Mild (↓)		
HER2/neu					Moderate (↑)							Moderate (↑)	Very High (↑)																	High (↑)																			
HE4	Moderate (↑)	Mild (↑)			High (↑)	High (↑)	High (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	N/A	N/A							High (↑)	Moderate (↑)									High (↑)									High (↑)	High (↑)	High (↑)								
IL8																																																	
NGAL									Moderate (↑)			Mild (↑)	Moderate (↑)		Moderate (↑)																													Moderate (↑)					
NMP22												Mild (↑)	Moderate (↑)	Moderate (↑)																																			
NSE	Moderate (↑)	Moderate (↑)			Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	High (↑)																	High (↑)									High (↑)	High (↑)	High (↑)				Moderate (↑)	Moderate (↑)		High (↑)	
ProGRP	High (↑)	High (↑)			High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	High (↑)	Very High (↑)														Moderate (↑)	Moderate (↑)		High (↑)									High (↑)	High (↑)	High (↑)			High (↑)					
Total PSA	Moderate (↑)	Very High (↑)		Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Very High (↑)	Moderate (↑)	Moderate (↑)																																				High (↑)
fPSA													Moderate (↑)																																				
sAxl																												Moderate (↑)																					
SCC	Moderate (↑)	Moderate (↑)		Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	Moderate (↑)	N/A	Moderate (↑)	Moderate (↑)	N/A	N/A			Moderate (↑)				High (↑)	Moderate (↑)									High (↑)										High (↑)	High (↑)							High (↑)	
SEPT9																																																	
SHOX2																																																	
S100	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	Mild (↑)	N/A	N/A																	Very High (↑)															Moderate (↑)	Moderate (↑)		High (↑)	
8-OHdG															Moderate (↑)					High (↑)	Moderate (↑)																							High (↑)					

Table 2. Newly developed criteria that results from an extensive review from the Barcelona Criteria. The development of these new criteria is still ongoing, and they include all the knowledge derived from the exhaustive investigation performed during 8 years and the findings obtained during the whole process. These new criteria are still not closed to these days, and they will not be presented to the public until the corresponding publication will be reviewed and accepted. These new criteria are based on a higher number of comorbidities that can cause FP results in TM, in comparison with the Barcelona Criteria. TM: tumor marker; Comor.: comorbidities; Asc.: ascites, BPH: benign prostatic hyperplasia, Col.: colecistitis, Cho.: choleostasis, CLD: chronic liver disease, Mod. Ja.: moderate jaundice (total bilirubin >= 4 & total bilirubin > 8 mg/dl), Acu. Jau.: acute jaundice (bt >= 8 mg/dl), Pan.: pancreatitis, Pem./Pso.: pemphigus / psoriasis, Per/Ple Eff.: pericardial / pleural effusions, Pro: prostatitis, CKD: chronic kidney disease, KF: kidney failure, UTI: urinary tract infections, DM: diabetes mellitus, Sar: sarcoidosis, CD: celiac disease, HypoT.: hypothyroidism, HyperT.: hyperthyroidism, End.: endometriosis, OGBC: gynecologycal benign conditions, Preg.: pregnancy, Lip.: lipemia, Hem.: hemolysis, Syn.: syndromes (Crigler-Najjar, Gilbert, Dubin-Johnson, Rotor), ALD: alcoholic liver disease, Hep.: hepatitis (acute, chronic, alcoholic), Cirr.: hepatic cirrhosis, PBC: primary biliary cholangitis, HBD.: hepatobiliary diseases, AD: Addison disease, CS: Cushing syndrome, HyperPTH.: hyperparathyroidism, IBD: inflammatory bowel disease, CD: Crohn's disease, HPAFP: hereditary persistence of alfa fetoprotein, ND: neurodegenerative disease, RA: rheumatoid arthritis, PS: pulmonary fibrosis, TBC: tuberculosis, OPA: other pulmonary abnormalities, CVD: cardiovascular diseases, CAD: coronary artery disease, OIGD: other inflammatory/gi disease, ASCI: acute spinal cord injury, CLE: chronicle lead exposure, Ane.: anemia (due to iron deficiency), OAD: other autoimmune diseases, Medi.: pre-analitycal, iatrogenic medicines' intake. Comments: albumin increase only characteristic of dehydration, diarrea; hypoalbuminemia can be found in renal disease, enteropathy, burns, malnutrition and infection; since both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are not exclusively hepatic enzymes, it justifies that their increases are not specific of hepatic diseases in many cases —mainly if only the AST is elevated—, which happens in acute myocardial infarction; ALT use to be higher than AST in alcoholic hepatitis and cirrhosis, acute hepatitis, chronic hepatitis, obstructive jaundice, mononucleosis and cholestasis; increases of the serum activity of gamma-glutamyl transferase (GGT) has been observed in patients with neoplastic hepatic invasion, but normal ultrasound (US); in the Crigler-Najjar syndrome the unconjugated bilirubin increases; in Dubin-Johnson syndrome the unconjugated bilirubin also increases; in Rotor disease the conjugated bilirubin increases; sometimes, in cholestasis hepatic enzymes increase, but not the bilirubin (dissociated cholestasis) —this is characteristic of some phases of primary biliary cirrhosis or medications-induced cholestasis—; hypercalcemia is one of the metabolic complications more frequently found in malignant tumors, nonetheless, in prostate cancer it is found when metastasis has occurred; hereditary persistence of alfa-fetoprotein (AFP) can be a rare cause for AFP increases; AFP can increase in serum and amniotic fluid when there are genetic abnormalities —mainly neurological and in fetal bowel obstruction—, nonetheless, a decrease can also be found —mainly related with an increased risk of Down syndrome—; AFP can increase in serum in adults with ataxia telangiectasia non-classical (A-T variant) and ataxia with oculomotor apraxia type 2 (AOA2); CA 19.9 can increase in bronchiectasis, bronchiolitis or emphysema; CA 125 can also increase in menstruation, pelvic inflammatory disease, pelvic infection or benign ovarian cysts; CA 125 can increase in chronic obstructive pulmonary disease (COPD), acute pulmonary thromboembolism (PT) —increases are correlated with the severity of the disease—.

OBJECTIVES

- To check whether the new learnings achieved related to the **Barcelona Criteria** (proposed by the **Spanish Society of Laboratory Medicine (SEQC^ML)**, published first in 1994 by Molina et al.[9], and reviewed later in 2024 by Trapé et al.[11]), regarding the correct interpretation of TM and the elimination of false positives (FP), could achieve an acceptable algorithm performance in real patients.
- To validate whether the resulting algorithm could be used as a **non-invasive (only blood and urine) test for Multi-Cancer Early Detection (MCED) screening**. If achieved, use these results to help the medical community to understand how **Evidence-Based Laboratory Medicine (EBLM)** and new technologies —mainly machine learning (ML) algorithms (also known as AI-powered diagnostic tools), based in large and quality datasets—, can **help healthcare professionals to improve diagnosis accuracy, reduce medical errors and misdiagnoses, as well as avoiding invasive procedures**.
- To fine-tune the final details of our MCED algorithm as a preliminary step to the upcoming **multicenter, international clinical trial of 26,000 patients** that will be performed from March 2025 to December 2026 (still recruiting hospitals and medical centers to join the trial).
- To check whether the different modules included in the MCED algorithm related to **non-malignant diseases that affect the values of different TM by resulting in FP, may help in cancer prevention, since many of them are well-known risk factors for cancer and can predispose patients to it if they are not reverted early**. This is **specially relevant for those diseases related to overweight, obesity and metabolic syndrome (MetS)**, as they are closely linked to cancer causes[5].

METHODS

This study builds upon the knowledge gained from the prior development of the algorithm for the detection of cancer of unknown primary (CUP) —a non-invasive blood and urine test to help in differential diagnosis of patients with one or more metastatic sites, but without the primary site identified—, and by adding other specific algorithms for the screening of different types of cancer. The latter have been presented and published at several prestigious scientific congresses, such as: **American Society of Clinical Oncology (ASCO)** 2022 (breast[15], colon[16]), European Society for Medical Oncology (ESMO) Gastrointestinal Cancers Congress 2024 (liver[17]), ESMO Gynecological Cancers Congress 2024 (ovary[18]), and International Association for the Study of Lung Cancer (IASLC) World Conference on Lung Cancer (WCLC) 2024 (lung[19]). Besides, it is also based on different clinical research studies carried out over the last eight years. Moreover, it is also based on the **Barcelona Criteria**, in order to improve its screening accuracy by avoiding FP[20–38].

Even though in the algorithm modeling set, several innovative and/or experimental TM —serum calprotectin, alfa-fetoprotein L3 fraction (AFP-L3), cystein-rich angiogenic inducer 61 (Cyr61), epidermal growth factor receptor (EGFR), Human kallikrein 2 (hK2), neutrophil gelatinase-associated lipocalin (NGAL), protein induced by vitamin K absence or antagonist-II (PIVKA-II), [-2]proPSA (p2PSA), soluble Axl (sAxl), and 8-Hydroxi-2'-deoxyguanosine (8-OHdG)—, were included, this study was developed only with the 15 most routinely used TM — alfa-fetoprotein (AFP), beta human chorionic gonadotropin (beta-hCG), carbohydrate antigen 15.3 (CA 15.3), carbohydrate antigen 19.9 (CA 19.9), carbohydrate antigen 72.4 (CA 72.4), carbohydrate antigen 125 (CA 125), carcinoembryonic antigen (CEA), cytokeratin 19 fragment 21-1 (CYFRA 21-1), human epidydymis 4 (HE4), neuron-specific enolase (NSE), pro-gastrin-releasing peptide (ProGRP), prostate specific antigen total (PSA total), prostate specific antigen free (PSA free), squamous cell carcinoma (SCC) antigen, and S100 protein (S100). Besides, the whole panel is based on many different specific panels which, in turn, include many other clinical analytes —**to meet the Barcelona Criteria in order to minimize FP**—, such as: hematology, antibodies, electrolytes, proteins, hepatic enzymes, hormones, vitamins, and other specific serum and urine analytes, as well as urinalysis.

To develop the algorithm, all data was computed firstly with the **Multi-Biomarker Disease Activity Algorithm (MBDAA)**[39] —developed by **Bioprognos** (Barcelona, Spain)—. Besides, a couple of new machine learning algorithms (ML) were also included, such as **Evidence-Based Laboratory Medicine Algorithm (EBLMA)**[40] and **Artificial Intelligence Recursive Algorithm (AIRA)**[41] —both developed by **Blueberry Diagnostics** (Barcelona, Spain)—, to improve both sensitivity and specificity. EBLMA is the algorithm that includes the **Barcelona Criteria** basis already upgraded with more knowledge regarding the correct interpretation of TM and the elimination of FP. EBLMA include several functions —sub-algorithms— to detect the following well-known conditions: iron transport and storage function diseases —iron deficiency and anemia (distinguishing between microcytic, normocytic, and macrocytic); cardiovascular disease (CVD) and atherosclerotic cardiovascular disease (ASCVD); pancreatic endocrine diseases —insulin resistance (IR), prediabetes, diabetes mellitus (DM)—; thyroid function diseases —primary, secondary and tertiary hypo and hyperthyroidism, both of non-autoimmune or autoimmune etiology—; parathyroid function and bone metabolism diseases —hypoparathyroidism and primary or secondary hyperparathyroidism, as well as osteopenia and osteoporosis—; vitamin metabolism diseases —vitamin B12 deficiency and insufficiency, and vitamin D deficiency and insufficiency—; hepatic function diseases —non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), liver fibrosis and cirrhosis—; renal function diseases — chronic kidney disease (CKD)—; electrolyte imbalance-related diseases —hypo or hypercalcemia, hypo or hyperkalemia, hypo or hyperphosphatemia, hypo or hypermagnesemia, hypo or hypernatremia, —; prostate diseases —benign prostatic hyperplasia (BPH), prostatitis and prostatic intraepithelial neoplasia (PIN)—; ovarian diseases —endometriosis and polycystic ovary syndrome (PCOS)—; and inflammatory response diseases —rheumatoid arthritis—. In the other hand, AIRA is an algorithm that computes in a recursive way all the values from 0 to 100% for both sensitivity and specificity to find the optimal cutoff values for low, moderate (moderate-low and moderate-high), and high-risk results. After that, **parallel approximations to optimize overall sensitivity (Se) were conducted**. Finally, **serial approximations to enhance specificity (Sp) were performed** —both powered by a tailor-made ML algorithm—.

Following the above mentioned process, we were able to determine an initial sensitivity and specificity of the algorithm with a training set of 192,090 patients. This set was very heterogenous, as it included results analyzed by different suppliers for the same laboratory determinations —Roche, Siemens Healthineers, Beckmann or Abbott, among others—, with their own systems and reference values. So, the variability and the potential biases of the results obtained by this way may be too high, thus affecting the quality of the results. In this way, to avoid these potential biases that can alter the results —the accuracy of the algorithm—, the present study was designed with one single laboratory —**Laboratorio Echevarne** (Sant Cugat del Vallès, Barcelona, Spain)—, and consisted in a randomized controlled trial (RCT) with a target sample size (n) of 300.

All patients included in the RCT were recruited through 3 private medical centres in Barcelona (Spain). Patients had to accept and sign the informed consent, the ethics comittee aprovement, and the test requisition form (TRF) —an example of the new TRF designed for the upcoming RCT that will be performed in the U.S. with **Empire City Laboratories** (Brooklyn, NY, US) is shown (**Figure 3**)—.

In the case of the present study, although target sample size was 300 patients, we managed to enroll 314 patients to allow some backup. However, according to the inclusion and exclusion criteria (**Figure 2**), 36 patients were initially excluded from the study, because they had ongoing clinically diagnosed pathologies, symptoms or signs, so the sample size dropped to 278 patients. From this new study population, 110 patients were excluded because they didn't show up at the clinical facility for any of the follow-up visits —this is a critical point to improve for the upcoming RCT, since the cost of each patient is very high and for this RCT almost a third of the patients already tested were lost.—, so the new n consisted of 168 patients. From this new n, 2 patients were excluded, because some of their laboratory parameters and/or clinical information were wrong or incorrect. Thus, **a final n of 166 patients was achieved**. In this final n, both genders (male and female) were equally represented (**Tables 3-9**) and the mean age of the participants was 53,1 years, being 54,7 (40 - 82) in the male population and 51,5 (40 - 77) in the female population (**Tables 3, 5**).

Patient's blood and urine samples were obtained from October 2021 to June 2023. Blood samples were obtained by peripheral venipuncture in all participants. All analytics were performed in **Laboratorio Echevarne**. After centrifugation of the blood samples, serum TM and all of other analytes were quantified. Complete blood count (CBC) and erythrocyte sedimentation rate (ESR) were determined by Yumizen analyzers (**Horiba**, Kyoto, Japan). Serum antibodies were determined by Atellica analyzers (**Siemens**, Munich, Germany), but the antinuclear antibodies (ANA) were analyzed by Elecsys systems (**Roche**, Rotkreuz, Switzerland) and the Helicobacter pylori IgG were analyzed by Vidas systems (**bioMérieux**, Marcy-L'Étoile, France). The serum electrolytes and proteins were all determined by the Atellica analyzers. All the serum hepatic enzymes and hormones were also analyzed by Atellica systems. In the case of TM, AFP, beta-hCG, PSA total, and PSA free were analyzed by Atellica. CA 72.4, HE4, and ProGRP were analyzed by Elecsys. CA 15.3, CA 19.9, CA 125, CEA, CYFRA 21-1, and SCC were all analyzed by Alinity systems (**Abbott**, Chicago, IL, US). NSE and S100 were analyzed by Liason systems (**Diasorin**, Saluggia, Italy). The remaining serum and urine analytes were also determined by Atellica systems. The majority of the tests were performed within two days after samples were obtained —CA 72.4, ProGRP, and S100 were performed within a week after samples were obtained since these techniques were analyzed once a week—. On the other hand, novel TM were only analyzed in the training set and determined by: µTASWako™ i30 (**Fujifilm Medical Systems**, Tokyo, Japan), in the case of AFP-L3 and PIVKA-II; and Hybritech Access (**Beckman Coulter**, Brea, CA, US), in the case of hK2 and p2PSA. The rest of TM only used in the training set were analyzed by commercial enzyme-linked immunosorbent assay (ELISA) kits, from several suppliers, such as: **Bühlmann** (Schönenbuch, Switzerland), in the case of serum calprotectin; **Abcam** (Cambridge, UK), in the case of Cyr61 and EGFR; R&D Systems (**Bio-Techne**, Minneapolis, MN, US), in the case of NGAL and sAxl; and **Cayman Chemical** (Ann Arbor, MI, US), in the case of 8-OHdG.

Once all results were obtained, all those that exhibited abnormal values (that is results that were outside their reference ranges) were reprocessed, to make sure that they were not obtained due to technical errors and were real and potentially pathological. When all laboratory determinations were finished per each patient, they were processed by the last version of the algorithm giving their final result.

Several calculations were performed, mainly those related with atherogenic indices (**Table 6**), hepatic and billiary tract indices (**Table 4**), pancreatic endocrine indices (**Table 7**), and renal indices (**Table 8**). Besides, a few inflammatory ratios (**Table 9**) were also performed —many studies have suggested their use as a novel tool in the assessment of many malignancies—. We selected the ones that were **Evidence-Based Laboratory Medicine (EBLM)**. In this way, performed atherogenic indices were: atherogenic coefficient (AC)[42,43], atherogenic index of plasma (AIP)[43–45], Castelli risk index I[46,47], Castelli risk index I + bilirubin[47], Castelli risk index II[47], Castelli risk index II + bilirubin[47], cholesterol/bilirubin ratio[47], lipoprotein combine index (LCI)[42], lipid tetrad index (LTI)[48,49], lipid pentad index (LPI)[49,50], and triglycerides/HDL-c index[42]. Besides, pancreatic endocrine indices performed were: adult treatment panel III (ATP-III)[51,52] —metabolic syndrome—, triglycerides and glucose (TyG) index[53] —insulin resistance—, homeostatic model assessment (HOMA-IR)[54,55], homeostatic model assessment 2 (HOMA-2)[56], and quantitative insulin sensitivity check index (QUICKI)[55]. Moreover, hepatic and billiary tract indices performed were: fatty liver index (FLI)[57,58], hepatic steatosis index (HSI)[58,59], K-NALFD score[60], NAFLD liver fat score (LFS)[58], NAFLD logit score (NLS)[61], NAFLD Ridge score (NRS)[61], and ALD NAFLD index (ANI)[62] for the assessment of non-alcoholic fatty liver disease (NAFLD); activity NASH (acNASH)[63], ferritin-AST&ALT-platelets (FAT) score[64], Gholam score[65], and hypertension-ALT-insulin resistance (HAIR) score[66] for the assessment of non-alcoholic steatohepatitis (NASH); AST-to-platelet ratio index (APRI)[67,68], BMI-age-ALT-triglycerides (BAAT) score[68], BMI-AST/ALT ratio-diabetes (BARD) score[68,69], FIB 4 score[68,70], Fibrometer[71], Forns fibrosis index (FFI)[72], NAFLD fibrosis score (NFS)[68,69,73], and steatosis-associated fibrosis estimator (SAFE) score[74] for the assessment of liver fibrosis and cirrhosis. R Factor[75], and chronic liver disease (CLivD) score[76] were also performed for further risk-assessment. Furthermore, inflammatory ratios performed were: RDW-to-LYM[77], RDW-to-NEU[77], RDW-to-MON[77], RDW-to-RBC[77], RDW-to-PLT77[X], RDW-to-PDW[77], Hb-to-RDW[78], LYM-to-MON[79], MON-to-LYM[77,80], MON-to-NEU[77], MPV-to-LYM[81], MPV-to-NEU77[X], MPV-to-MON[77], MPV-to-PLT[77], NEU-to-LYM[77,82], Derived NEU-to-LYM[77,83], NEU-to-LYM+PLT[84], NEU-to-MON[77,85], PDW-to-LYM[86], PDW-to-NEU[77], PLT-to-WBC[87], PLT-to-LYM[80,88], PLT-to-NEU[89], PLT-to-MON[77,90], CRP-to-RDW[91], ALP-to-RBC[77], ALP-to-RDW[77], ALP-to-PLT[77,92], ALP-to-PDW[77], LDH-to-RBC[77], LDH-to-RDW77[X], LDH-to-LYM[77,93], LDH-to-PLT[77,93], LDH-to-PDW ratio[77], LDH-to-albumin[93], NEU-to-HDL-c[94], MON-to-HDL-c[94,95], PLT-to-HDL-c[95,96], prognostic nutritional index (PNI)[97], CA 15.3-to-RBC[77], CA 15.3-to-RDW[77], CA 15.3-to-PLT[77], and CA 15.3-to-PDW[77]. Finally, renal indices performed were: corrected electrolytes[98–100], creatinine clearance[101], eGFR[102], BUN-to-creatinine ratio[103], and urine albumin-to-creatinine ratio (ACR)[104]. Then, the cancer-related functions of the algorithms were executed.

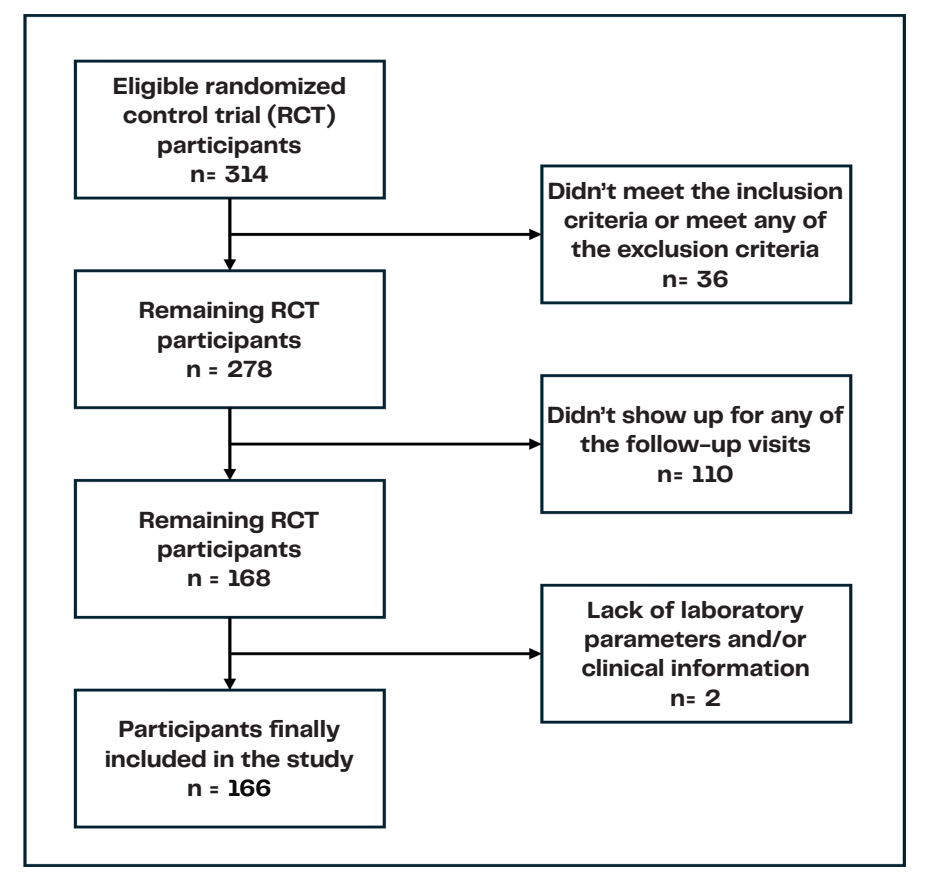
In turn, all patients with suspicious findings were referred to the corresponding medical centres for confirmation and subsequent classification in each of the groups —case and control—, designed for the biostatistics upcoming phase. Patients attended follow-up visits in one year.

Inclusion Criteria

- Men and women with not known currently clinically diagnosed pathologies, without symptoms or signs, white/caucasian ethnicity, aged 40 years, onwards; and
- Patients whose high anthropometric indicators and/or lifestyle habits may predispose them to cancer:
  - Body mass index (BMI), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR), hypertriglyceridemic waist (HW), visceral adiposity index (VAI) —which are indicative of overweight and obesity—; and/or or
  - High red/processed meat consumption, low fruits and vegetables intake, lack of physical activity, smoking habits, and/or alcohol intake.

Exclusion Criteria

- Patients who didn't meet the inclusion criteria or meet any of the following criteria:
  - Patients that didn't show up at the clinical facility in any of the follow-up visits.
  - Patients lacking laboratory parameters and/or clinical information.



Eligible randomized control trial (RCT) participants n= 314

Didn't meet the inclusion criteria or meet any of the exclusion criteria n= 36

Remaining RCT participants n = 278

Didn't show up for any of the follow-up visits n= 110

Remaining RCT participants n = 168

Lack of laboratory parameters and/or clinical information n= 2

Participants finally included in the study n = 166

Figure 2. Inclusion and exclusion criteria for the selection of the study population, as well as the graphical flow of the patients that were selected in the final study population.

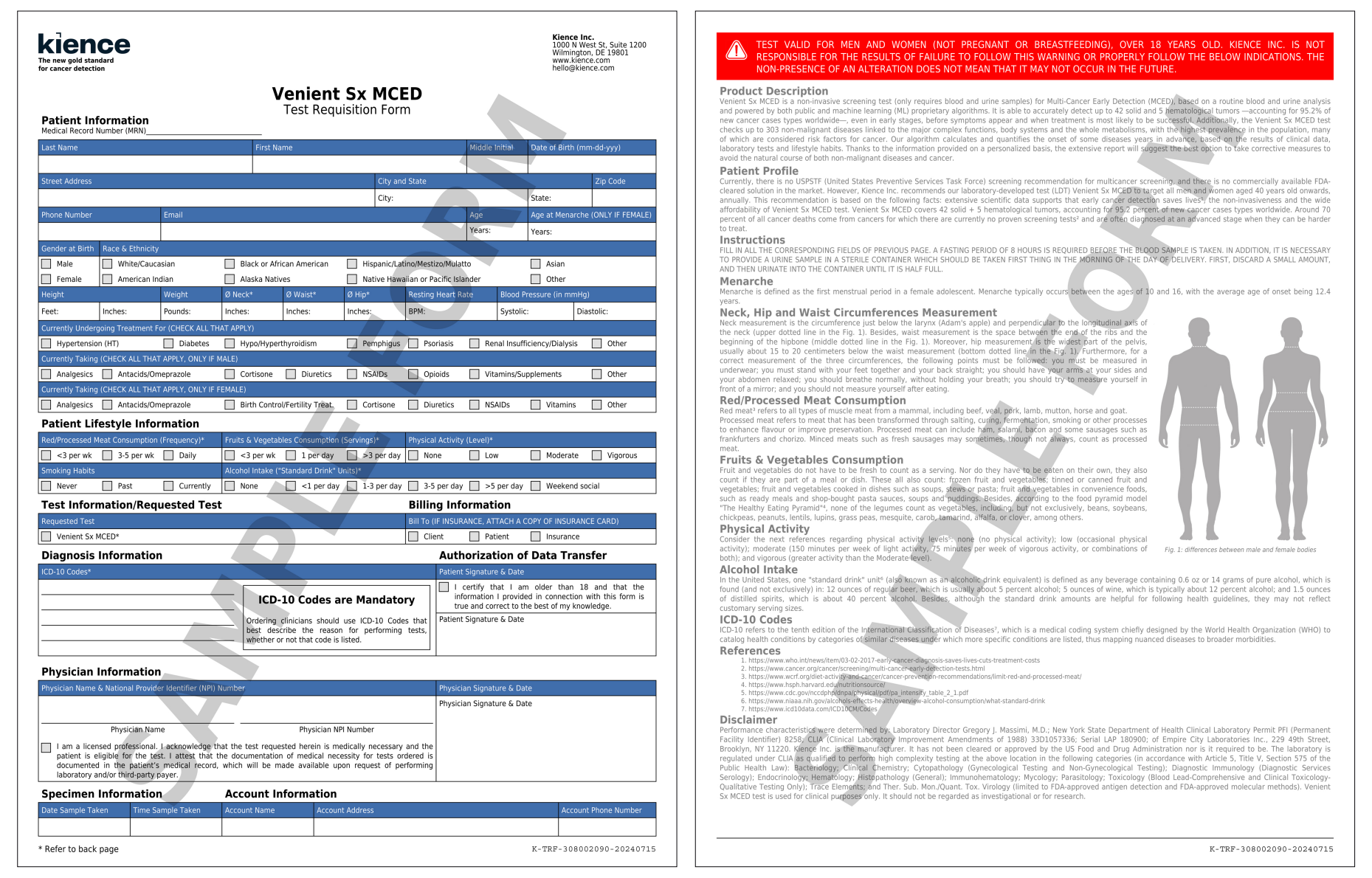


Figure 3. Test requisition form (TRF) for the RCT to be performed in the U.S. (Empire City Laboratories Inc., Brooklyn, NY, U.S.). This new RCT will be based in a sample size (n) of 1,000 patients —paying special attention to the proportion of different ethnicities of the population of the U.S, in order to achieve an n as realistic as possible—. The different anthropometric data and lifestyle habits needed to perform the algorithm are displayed in the TRF.

Parameter	Total	Male	Female
Age (y.o.)	53,1 (40 - 82)	54,73 (30 - 82)	70,1 (40 - 77)
Height (cm)	170,02 (150 - 190)	176,42 (152 - 190)	163,62 (150 - 177)
Weight (kg)	71,36 (38,6 - 128)	82,95 (38,6 - 128)	59,77 (45 - 112)
Neck Circumference (cm)	36,48 (28 - 50)	40,21 (30 - 47)	32,75 (28 - 50)
Waist Circumference (cm)	86,34 (62,5 - 131)	96,73 (68,5 - 131)	75,95 (62,5 - 115)
Hip Circumference (cm)	97,33 (70 - 127)	100,3 (70 - 119)	94,36 (75 - 127)
Menarche (y.o.)	NA	NA	12,81 (9 - 17)
Systolic Pressure (mmHg)	117,28 (77 - 170)	122,57 (95 - 170)	111,99 (77 - 148)
Diastolic Pressure (mmHg)	74 (43 - 109)	77,24 (53 - 109)	70,76 (43 - 93)
Resting Heart Rate (BPM)	69,03 (42 - 110)	68,35 (42 - 110)	69,71 (55 - 99)
Red Blood Cell Count (*10^12/L)	4,49 (3,07 - 5,64)	4,76 (4,07 - 5,64)	4,22 (3,07 - 5,49)
Hemoglobin (g/dL)	13,74 (7,1 - 17,3)	14,6 (7,1 - 17,3)	12,88 (9,3 - 15,8)
Hematocrit (%)	40,94 (23,3 - 51)	43,38 (23,3 - 51)	38,49 (29,3 - 47,7)
HCT/Hb	2,99 (2,79 - 4,69)	2,99 (2,79 - 4,69)	2,99 (2,82 - 3,6)
ESR (mm)	6,41 (2 - 38)	6,01 (2 - 33)	6,82 (2 - 38)
MCV (fL)	90,92 (9,2 - 107,37)	90,26 (9,2 - 107,37)	91,57 (70,77 - 106,6)
MCH (pg)	30,77 (16,36 - 35,63)	30,84 (16,36 - 35,63)	30,7 (22,46 - 34,52)
MCHC (g/dL)	33,65 (30,47 - 36,22)	33,76 (30,47 - 36,22)	33,53 (31,74 - 35,44)
RDW (%)	14,25 (11,7 - 21,9)	14,36 (11,9 - 21,9)	14,13 (11,7 - 19,8)
Leukocytes Absolute (x10^9/L)	5,61 (2,52 - 11,94)	5,96 (2,96 - 11,94)	5,25 (2,52 - 9,77)
Neutrophils Absolute (x10^9/L)	3,34 (1,11 - 9,64)	3,56 (1,11 - 9,64)	3,13 (1,31 - 7,43)
Lymphocytes Absolute (x10^9/L)	1,63 (0,35 - 2,89)	1,7 (0,76 - 2,72)	1,56 (0,35 - 2,89)
Monocytes Absolute (x10^9/L)	0,43 (0,13 - 0,98)	0,48 (0,23 - 0,98)	0,39 (0,13 - 0,76)
Eosinophils Absolute (x10^9/L)	0,17 (0,02 - 1,01)	0,2 (0,02 - 0,61)	0,15 (0,03 - 1,01)
Basophils Absolute (x10^9/L)	0,03 (0 - 0,09)	0,03 (0 - 0,09)	0,03 (0 - 0,07)
Neutrophils Ratio (%)	58,39 (32,6 - 80,7)	58,3 (32,6 - 80,7)	58,49 (40,6 - 77,4)
Lymphocytes Ratio (%)	30,25 (8,9 - 58)	29,66 (8,9 - 58)	30,83 (12,5 - 48,2)
Monocytes Ratio (%)	7,72 (4 - 12,8)	8,07 (4,4 - 11,9)	7,37 (4 - 12,8)
Eosinophils Ratio (%)	3,07 (0,4 - 12,3)	3,36 (0,4 - 10,3)	2,77 (0,4 - 12,3)
Basophils Ratio (%)	0,57 (0,1 - 1,8)	0,61 (0,1 - 1,8)	0,53 (0,1 - 1,4)
Platelet Count (x10^9/L)	239,47 (136 - 442)	239,39 (136 - 442)	239,55 (143 - 372)
MPV (fL)	9,45 (6,7 - 14)	9,49 (8 - 14)	9,41 (6,7 - 12,8)
PDW (%)	16,39 (8,2 - 30,4)	16,56 (8,2 - 26,4)	16,21 (9,2 - 30,4)
PT (%)	92,98 (24 - 100)	91,24 (24 - 100)	94,71 (67 - 100)
aPTT (s)	31,22 (24,6 - 52,4)	31,53 (25,2 - 52,4)	30,92 (24,6 - 38,1)
Calcium (mg/L)	96,6 (85,6 - 127,3)	96,77 (86,3 - 106,8)	96,43 (85,6 - 127,3)
Chloride (mmol/L)	105,89 (98 - 110)	105,52 (98 - 110)	106,27 (103 - 110)
Magnesium (mg/dL)	1,98 (1,6 - 2,97)	2,02 (1,75 - 2,97)	1,94 (1,6 - 2,52)
Phosphorus (mg/dL)	3,47 (1,9 - 6,7)	3,37 (2,2 - 4,7)	3,57 (1,9 - 6,7)
Potassium (mmol/L)	4,23 (3,42 - 5,21)	4,31 (3,42 - 5,21)	4,16 (3,51 - 4,98)
Sodium (mmol/L)	139,97 (131,8 - 147)	140,14 (131,8 - 147)	139,79 (135,3 - 147)
Albumin (g/L)	45,31 (31 - 52)	45,27 (31 - 52)	45,36 (40 - 50)
Apolipoprotein A1 (mg/dL)	146,96 (93 - 230)	135,72 (93 - 230)	158,2 (109 - 220)
Apolipoprotein B (mg/dL)	88,92 (50 - 179)	96,25 (56 - 179)	81,58 (50 - 133)
CRP-hs (mg/L)	2,93 (0,15 - 72,27)	4,17 (0,15 - 72,27)	1,68 (0,15 - 17,47)
Ferritin (ng/mL)	97,91 (3,3 - 1743,6)	147,92 (3,6 - 1743,6)	47,91 (3,3 - 330,7)
Globulin (g/L)	26,03 (18 - 39)	25,95 (18 - 39)	26,1 (19 - 37)
Lipoprotein (a) (mg/dL)	32,21 (9,99 - 158,58)	40 (9,99 - 158,58)	24,42 (9,99 - 112,04)
Total Protein (g/L)	71,34 (59 - 83)	71,21 (59 - 81,1)	71,47 (64 - 83)
ALP (IU/L)	66,9 (30 - 311)	68,55 (39 - 311)	65,25 (30 - 311)
AST (IU/L)	24,38 (12 - 125)	27,18 (12 - 125)	21,58 (13 - 72)
ALT (IU/L)	24,16 (7 - 114)	28,49 (8 - 110)	19,83 (7 - 114)
GGT (IU/L)	27,33 (6,99 - 199)	31,51 (10 - 167)	23,14 (6,99 - 199)
LDH (IU/L)	166,34 (114 - 553)	174,81 (114 - 553)	157,88 (116 - 206)
Total Cholesterol (mg/dL)	193,24 (120 - 302)	194,4 (128 - 302)	192,08 (120 - 268)
Cholesterol HDL (mg/dL)	60,33 (31 - 95)	50,22 (31 - 95)	70,43 (42 - 95)
Cholesterol non-HDL (mg/dL)	132,92 (53 - 265)	144,18 (53 - 265)	121,65 (53 - 185)
Cholesterol LDL (mg/dL)	114,74 (35,4 - 223,49)	122,11 (35,4 - 223,49)	107,37 (35,4 - 173)
Cholesterol VLDL (mg/dL)	18,18 (6,6 - 74,44)	22,07 (6,8 - 74,44)	14,28 (6,6 - 32,66)
Triglycerides (mg/dL)	96,54 (33 - 603)	121,12 (34 - 603)	71,96 (33 - 209)
Vitamin B 12 (pg/mL)	418,42 (199 - 1092)	406,28 (199 - 1084)	430,55 (240 - 1092)
Vitamin D 25-OH (ng/mL)	28,63 (12,03 - 104,18)	26,71 (12,03 - 104,18)	30,55 (12,77 - 88,29)
Anti-CCP ab (U/mL)	0,72 (0,43 - 10,3)	0,77 (0,43 - 10,3)	0,68 (0,49 - 4,1)
H. pylori IgG Ab. (U/mL)	1,85 (0,32 - 8,01)	2,09 (0,32 - 8,01)	1,61 (0,39 - 8,01)
Rheumatoid Factor (UI/mL)	8,54 (3,99 - 210)	11,78 (3,99 - 210)	5,31 (3,99 - 12)
FSH (mIU/mL)	NA (0,29 - NA)	(-)	42,8 (0,29 - 175,54)
Insulin (µU/mL)	7,65 (1,37 - 37,05)	7,87 (1,37 - 26,73)	7,42 (3,01 - 37,05)
PTHi (pg/mL)	41,83 (7,2 - 140,6)	43,26 (14,4 - 85)	40,41 (7,2 - 140,6)
TSH (mIU/L)	2,54 (0,22 - 40,22)	2,29 (0,27 - 40,22)	2,79 (0,22 - 40,22)
T4 Free (ng/dL)	1,04 (0,63 - 1,7)	1,06 (0,63 - 1,56)	1,02 (0,63 - 1,7)
AFP (ng/mL)	2,95 (1,09 - 16,4)	2,94 (1,09 - 16,4)	2,96 (1,09 - 15,3)
Beta-hCG (mIU/mL)	517,19 (1,99 - 85483,2)	2,01 (1,99 - 2,7)	1032,36 (1,99 - 85483,2)
CA 15.3 (U/mL)	19,04 (2,6 - 137,3)	14,36 (3,12 - 33,6)	23,72 (2,6 - 137,3)
CA 19.9 (U/mL)	9,91 (1,19 - 96,93)	40,06 (1,19 - 96,93)	10,79 (1,19 - 60,97)
CA 72.4 (U/mL)	7,2 (0,49 - 250,01)	8,22 (0,49 - 250,01)	6,19 (0,49 - 234,5)
CA 125 (U/mL)	14,12 (2,5 - 82,4)	10,81 (2,5 - 38,4)	17,43 (2,6 - 82,4)
CEA (ng/mL)	1,54 (0,39 - 4,3)	1,6 (0,49 - 4,3)	1,47 (0,39 - 3,15)
CYFRA 21-1 (ng/mL)	2,17 (0,02 - 78,48)	2,89 (0,02 - 78,48)	1,45 (0,37 - 7,34)
HE4 (pmol/L)	36,19 (14,1 - 80)	32,9 (32,9 - 32,9)	36,23 (14,1 - 80)
NSE (ng/mL)	10,59 (5,4 - 41,66)	11,34 (5,48 - 41,66)	9,85 (5,4 - 19,79)
ProGRP (pg/mL)	30,22 (6,6 - 59,8)	28,49 (6,6 - 59,8)	31,98 (11,7 - 56,4)
Total PSA (ng/mL)	1,17 (0 - 12,86)	1,42 (0,07 - 12,86)	NA
Free PSA (ng/mL)	0,32 (0 - 1,39)	0,36 (0,01 - 1,39)	NA
SCC (ng/mL)	1,11 (0,4 - 3)	1,19 (0,5 - 2,9)	1,03 (0,4 - 3)
S100 (ng/mL)	0,07 (0 - 1,91)	0,09 (0,01 - 1,91)	0,05 (0 - 0,17)
Creatinine (mg/dL)	0,89 (0,56 - 1,78)	0,99 (0,62 - 1,78)	0,8 (0,56 - 1,74)
Glucose (mg/dL)	94,86 (74 - 142)	96,57 (75 - 142)	93,14 (74 - 125)
HbA1c (%)	5,28 (3,6 - 7,2)	5,36 (3,6 - 7,2)	5,21 (4,4 - 6,2)
Serum Iron (µg/dL)	91,08 (13 - 237)	92,58 (21 - 206)	89,59 (13 - 237)
Total Bilirubin (mg/dL)	0,72 (0,22 - 1,98)	0,76 (0,26 - 1,72)	0,67 (0,22 - 1,98)
Direct Bilirubin (mg/dL)	0,23 (0,09 - 0,65)	0,24 (0,09 - 0,58)	0,22 (0,09 - 0,65)
Indirect Bilirubin (mg/dL)	0,49 (-0,43 - 1,36)	0,52 (0,17 - 1,36)	0,46 (-0,43 - 1,35)
Urea (mg/dL)	35,68 (17 - 62)	37,23 (19 - 62)	34,13 (17 - 51)
BUN (mg/dL)	16,65 (7,93 - 28,93)	17,37 (8,87 - 28,93)	15,93 (7,93 - 23,8)
Uric Acid (mg/dL)	5,12 (2,1 - 10,3)	5,98 (2,7 - 10,3)	4,26 (2,1 - 7,7)
Urine Albumin (g/L)	0,11 (0,04 - 0,82)	0,12 (0,04 - 0,36)	0,11 (0,04 - 0,82)
Urine Creatinine (g/L)	1,36 (0,21 - 3,92)	1,53 (0,21 - 3,92)	1,19 (0,35 - 3,42)
Urine Density (g/L)	1020,21 (1007 - 1034)	1020,65 (1008 - 1032)	1019,77 (1007 - 1034)
Urine pH	5,77 (5 - 7,5)	5,86 (5 - 7,5)	5,69 (5 - 7,5)
Urine Leukocytes	0,37 (0 - 3)	0,07 (0 - 3)	0,66 (0 - 3)
Urine Nitrites	0,04 (0 - 1)	0,02 (0 - 1)	0,05 (0 - 1)
Urine Protein	0,05 (0 - 2)	0,04 (0 - 2)	0,06 (0 - 2)
Urine Glucose	0,01 (0 - 2)	0,02 (0 - 2)	0 (0 - 0)
Urine Ketones	0,07 (0 - 3)	0,06 (0 - 3)	0,07 (0 - 3)
Urine Urobilinogen	0,04 (0 - 2)	0,05 (0 - 2)	0,02 (0 - 2)
Urine Urobilin	0 (0 - 0)	0 (0 - 0)	0 (0 - 0)
Urine Red Blood Cells	0,1 (0 - 3)	0,06 (0 - 3)	0,13 (0 - 2)
Urine Hemoglobin	0,1 (0 - 3)	0,06 (0 - 3)	0,13 (0 - 2)

Table 3. Raw data —clinical and laboratory data—, obtained, displayed as the mean value of each parameter with the corresponding minimal (Min) and maximal (Max) value, for all patients and by gender: male and female.

Parameter	Total	Male	Female
Fatty Liver Index (FLI)	30,38 (1,18 - 97,33)	45,68 (2,9 - 97,33)	15,08 (1,18 - 88,52)
Hepatic Steatosis Index (HSI)	33,3 (21,45 - 49,46)	35,14 (21,45 - 49,46)	31,47 (22,87 - 48,97)
K-NAFLD	-1,88 (-6,05 - 7,86)	-0,56 (-5,63 - 7,33)	-3,2 (-6,05 - 7,86)
NAFLD Liver Fat Score (LFS)	-1,5 (-3,36 - 2,86)	-1 (-2,91 - 2,86)	-2,02 (-3,36 - 2,49)
NAFLD Logit Score (NLS)	0,17 (0 - 1)	0,31 (0 - 1)	0,03 (0 - 1)
NAFLD Ridge Score (NRS)	-7,23 (-16,79 - 25,06)	-3,68 (-15,16 - 25,06)	-10,78 (-16,79 - 2,46)
ALD NAFLD Index (ANI)	-2,8 (-54,3 - 11,19)	-1,12 (-54,3 - 11,19)	-4,49 (-16,73 - 5,23)
Total NAFLD Risk	0,94 (0 - 3,2)	1,47 (0 - 3,2)	0,4 (0 - 2,4)
Activity NASH (acNASH)	3,16 (1,28 - 14,57)	3,17 (1,28 - 14,57)	3,15 (1,52 - 12,15)
Ferritin-AST&ALT-Platelets (FAT) Score	0,22 (0 - 3)	0,36 (0 - 3)	0,08 (0 - 2)
Gholam Score	8,21 (6,74 - 12,68)	8,47 (7,11 - 12,68)	7,96 (6,74 - 11,23)
HT-ALT-Insulin Resistance (HAIR)	0,81 (0 - 3)	1,17 (0 - 3)	0,43 (0 - 2)
Total NASH Risk	0,51 (0 - 2,75)	0,65 (0 - 2,75)	0,38 (0 - 2)
AST-to-Platelet Ratio Index (APRI)	0,31 (0,1 - 1,08)	0,34 (0,1 - 1,08)	0,28 (0,13 - 0,81)
BMI-Age-ALT-Triglycerides (BAAT)	0,87 (0 - 3)	1,2 (0 - 3)	0,54 (0 - 2)
BMI-AST/ALT Ratio-Diabetes (BARD)	1,42 (0,52 - 3,93)	1,53 (0,52 - 3,93)	1,32 (0,56 - 2,46)
Chronic Liver Disease (CLivD) Score	70,8 (18 - 181)	79 (21 - 155)	62,6 (18 - 181)
FIB 4 Score	1,2 (0,47 - 4,01)	1,27 (0,47 - 4,01)	1,13 (0,48 - 2,62)
Fibrometer	20,29 (16,89 - 25,81)	21,12 (17,73 - 25,81)	19,46 (16,89 - 24,6)
Forns Fibrosis Index (FFI)	4,2 (1,22 - 7,09)	4,42 (1,22 - 7,09)	3,99 (1,69 - 6,54)
NAFLD Fibrosis Score (NFS)	-2,25 (-5,67 - 0,62)	-2,01 (-5,67 - 0,62)	-2,5 (-5,45 - 0,55)
Steatosis-Associated Fibrosis Estimator (SAFE)	-50,95 (-203,41 - 197,88)	-28,91 (-192,01 - 197,88)	-72,99 (-203,41 - 132,73)
Total Fibrosis Risk	0,38 (0,13 - 1,88)	0,45 (0,13 - 1,88)	0,32 (0,13 - 1,25)
R factor	0,95 (0,12 - 5,17)	1,22 (0,15 - 5,17)	0,69 (0,12 - 2,92)

Table 4. Hepatic and billiary tract indices, displayed as the mean value of each parameter with the corresponding minimal (Min) and maximal (Max) value, for all patients and by gender: male and female.

Parameter	Total	Male	Female
Age (y.o.)	53,1 (40 - 82)	40 (40 - 82)	51,47 (40 - 77)
Height (cm)	170,02 (150 - 190)	152 (152 - 190)	163,62 (150 - 177)
Weight (kg)	71,36 (38,6 - 128)	38,6 (38,6 - 128)	59,77 (45 - 112)
Body Mass Index (BMI)	24,44 (15,94 - 36,6)	16,71 (16,71 - 36,6)	22,33 (-9,62 - 36,29)
Neck Circumference (cm)	36,48 (28 - 50)	30 (30 - 47)	32,75 (28 - 50)
Waist Circumference (cm)	86,34 (62,5 - 131)	68,5 (68,5 - 131)	75,95 (62,5 - 115)
Hip Circumference (cm)	97,33 (70 - 127)	70 (70 - 119)	94,36 (75 - 127)
Waist-to- Hip Ratio (WHR)	0,88 (0,7 - 1,17)	0,8 (0,8 - 1,17)	0,8 (0,7 - 1)
Deurenberg Body Fat (%)	30,75 (13,51 - 54,06)	13,51 (13,51 - 39,69)	33,23 (25 - 54,06)
Palafolls Body Fat (%)	32,28 (19,15 - 49,71)	19,15 (19,15 - 39,53)	35,26 (28,47 - 49,71)
Hodgdon Beckett Body Fat (%)	25,03 (8,49 - 54,61)	9,1 (9,1 - 38,7)	27,07 (8,49 - 54,61)
Body Fat Mass	18,56 (4,58 - 56,71)	5,34 (5,34 - 45,67)	17,31 (4,58 - 56,71)
Lean Body Mass	52,8 (33,26 - 82,5)	33,26 (33,26 - 82,5)	42,45 (34,59 - 64,28)
Ideal Body Fat Jackson Pollard	21,27 (15,3 - 26,3)	15,3 (15,3 - 20,9)	24,02 (22,2 - 26,3)
Body Fat To Lose To Ideal	3,66 (-9,62 - 30,25)	-8,58 (-8,58 - 24,51)	2,88 (-9,62 - 30,25)
Body Density	1,04 (0,98 - 1,08)	1,01 (1,01 - 1,08)	1,04 (0,98 - 1,08)
Waist-to-Height Ratio (WHtR)	0,51 (0,37 - 0,73)	0,41 (0,41 - 0,73)	0,47 (0,37 - 0,67)
Lipid Accumulation Product (LAP)	28,6 (2,75 - 202,98)	3,92 (3,92 - 202,98)	14,74 (2,75 - 77,48)
Body Adiposity Index (BAI)	26,01 (15,54 - 43,27)	18,27 (18,27 - 34,82)	27,19 (15,54 - 43,27)
Visceral Adiposity Index (VAI)	0,97 (0,2 - 7,58)	0,2 (0,2 - 7,58)	0,67 (0,25 - 2,95)
Body Shape Index (BSI)	0,08 (0,06 - 0,1)	0,07 (0,07 - 0,1)	0,07 (0,06 - 0,06)
Conicity Index (CI)	1,22 (0,94 - 1,53)	1,06 (1,06 - 1,53)	1,15 (0,94 - 1,37)

Table 5. Anthropometric indices and ratios, displayed as the mean value of each parameter with the corresponding minimal (Min) and maximal (Max) value, for all patients and by gender: male and female.

Parameter	Total	Male	Female
Triglycerides/HDL-c index	1,91 (0,42 - 16,75)	2,72 (0,49 - 16,75)	1,1 (0,42 - 4,86)
Atherogenic index of plasma (AIP)	-0,2 (-0,73 - 0,86)	-0,04 (-0,67 - 0,86)	-0,37 (-0,73 - 0,33)
Castelli risk I	3,45 (1,56 - 8,16)	4,09 (1,56 - 8,16)	2,82 (1,56 - 4,59)
Castelli risk I + bilirubin	1,57 (0,58 - 2,91)	1,67 (0,75 - 2,91)	1,47 (0,58 - 2,31)
Castelli risk II	2,1 (0,37 - 6,14)	2,6 (0,37 - 6,14)	1,61 (0,37 - 3,08)
Castelli risk II + bilirubin	0,94 (0,23 - 2,14)	1,05 (0,23 - 2,14)	0,83 (0,23 - 1,47)
Cholesterol/biilirubin ratio	3,24 (0,83 - 8,81)	3,08 (1 - 8,81)	3,4 (0,83 - 8,73)
Atherogenic coefficient (AC)	2,45 (0,56 - 7,16)	3,09 (0,56 - 7,16)	1,82 (0,56 - 3,59)
Lipoprotein combine index (LCI)	4,48 (3,66 - 5,62)	4,68 (3,69 - 5,62)	4,29 (3,66 - 4,95)
Lipid tetrad index (LTI)	3,77 (2,89 - 4,47)	4,03 (2,91 - 5,34)	3,51 (2,89 - 4,47)
Lipid pentad index (LPI)	5,31 (4,27 - 6,78)	5,56 (4,37 - 6,78)	5,06 (4,27 - 6,07)

Table 6. Atherogenic indices indices, displayed as the mean value of each parameter with the corresponding minimal (Min) and maximal (Max) value, for all patients and by gender: male and female.

Parameter	Total	Male	Female
ATP-III	1,58 (1 - 5)	1,82 (1 - 5)	1,34 (1 - 4)
TyG Index	8,27 (7,29 - 10,3)	8,5 (7,32 - 10,3)	8,04 (7,29 - 9,17)
HOMA-IR	1,86 (0,28 - 9,97)	1,93 (0,28 - 6,53)	1,78 (0,63 - 9,97)
HOMA-Beta	89,28 (21,6 - 289,96)	88,93 (21,6 - 267,3)	89,64 (35,9 - 289,96)
HOMA-S	94,17 (10,03 - 357,14)	103,14 (15,31 - 357,14)	84,82 (10,03 - 158,73)
HOMA-2	4,49 (4,01 - 5,5)	4,63 (4,01 - 5,5)	4,34 (4,09 - 4,7)
QUICKI	0,37 (0,28 - 0,49)	0,37 (0,29 - 0,49)	0,37 (0,28 - 0,42)

Table 7. Pancreatic endocrine indices, displayed as the mean value of each parameter with the corresponding minimal (Min) and maximal (Max) value, for all patients and by gender: male and female.

Parameter	Total	Male	Female
Serum Calcium	96,6 (85,6 - 127,3)	96,77 (86,3 - 106,8)	96,43 (85,6 - 127,3)
Corrected Calcium	92,35 (82,4 - 121,7)	92,56 (83,1 - 99,6)	92,14 (82,4 - 121,7)
Serum Chloride	105,89 (98 - 110)	105,52 (98 - 110)	106,27 (103 - 110)
Corrected Chloride	106,31 (99,34 - 114,06)	105,8 (99,34 - 114,06)	106,81 (103,38 - 111,11)
Serum Magnesium	1,98 (1,6 - 2,97)	2,02 (1,75 - 2,97)	1,94 (1,6 - 2,52)
Corrected Magnesium	1,95 (1,59 - 2,96)	1,99 (1,71 - 2,96)	1,91 (1,59 - 2,51)
Serum Sodium	139,97 (131,8 - 147)	140,14 (131,8 - 147)	139,79 (135,3 - 147)
Corrected Sodium	139,84 (131,9 - 146,95)	140,06 (131,9 - 146,69)	139,63 (135,08 - 146,95)
Calcium/Magnesium Ratio	4,92 (2,98 - 7,03)	4,84 (2,98 - 5,86)	5,01 (3,99 - 7,03)
Calcium/Phosphorus Ratio	2,85 (1,71 - 4,95)	2,92 (2,07 - 3,98)	2,79 (1,71 - 4,95)
Sodium/Potassium Ratio	33,23 (26,87 - 41,4)	32,71 (26,87 - 41,4)	33,75 (28,51 - 40,74)
Creatinine Clearance	90,99 (34,91 - 183,93)	101,14 (53,47 - 183,93)	80,85 (34,91 - 168,4)
Estimated Glomerular Filtration Rate (eGFR)	86,81 (33,62 - 122,35)	86,48 (45,7 - 122,35)	87,13 (33,62 - 113,69)
KFRE 4 North American 2	0,08 (0 - 122,35)	0,04 (0 - 1,57)	0,12 (0 - 4,34)
KFRE 4 North American 5	0,25 (0 - 12,95)	0,13 (0 - 4,82)	0,36 (0 - 12,95)
KFRE 4 non North American 2	0,05 (0 - 2,93)	0,03 (0 - 1,05)	0,08 (0 - 2,93)
KFRE 4 non North American 5	0,21 (0 - 10,87)	0,11 (0 - 4,02)	0,3 (0 - 10,87)
Urine ACR	114,66 (18,87 - 1714,29)	109,54 (18,87 - 1714,29)	119,85 (24,24 - 1368,93)

Table 8. Corrected electrolytes, renal indices and the Kidney Failure Risk Equation (KFRE) to determine 2 and 5 year probability of treated kidney failure —dialysis or transplantation— for a patient with CKD stage 3a, 3b, 4, or 5 —kidney failure (KF)—, displayed as the mean value of each parameter with the corresponding minimal (Min) and maximal (Max) value, for all patients and by gender: male and female.

Parameter	Total	Male	Female
RDW-to-LYM Ratio (RDWLR)	9,87 (4,98 - 43,45)	5,11 (5,11 - 28,18)	10,29 (4,98 - 43,45)
RDW-to-NEU Ratio (RDWNR)	5,03 (1,53 - 13)	1,53 (1,53 - 12,27)	5,34 (1,75 - 13)
RDW-to-MON Ratio (RDWMR)	38,64 (15,01 - 117,04)	15,01 (15,01 - 77,68)	43,56 (15,01 - 117,04)
RDW-to-RBC Ratio (RDWRBCR)	3,22 (2,39 - 5,05)	2,39 (2,39 - 5,05)	3,4 (2,62 - 4,89)
RDW-to-PLT Ratio (RDWPR)	0,06 (0,03 - 0,11)	0,03 (0,03 - 0,11)	0,06 (0,04 - 0,1)
RDW-to-PDW Ratio (RDWPDWR)	0,91 (0,44 - 2,12)	0,5 (0,5 - 2,12)	0,93 (0,44 - 1,61)
Hb-to-RDW Ratio (RDWR)	9,77 (3,24 - 12,75)	3,24 (3,24 - 12,75)	9,21 (4,7 - 11,53)
LYM-to-MON Ratio (LMR)	4,15 (1,09 - 11,05)	1,09 (1,09 - 8,66)	4,46 (1,64 - 11,05)
MON-to-LYM Ratio (MLR)	0,28 (0,09 - 0,92)	0,12 (0,12 - 0,92)	0,26 (0,09 - 0,61)
MON-to-NEU Ratio (MNR)	0,14 (0,06 - 0,28)	0,07 (0,07 - 0,25)	0,13 (0,06 - 0,28)
MPV-to-LYM Ratio (MPVLR)	6,39 (3,4 - 20,28)	3,4 (3,4 - 13,15)	6,64 (3,4 - 20,28)
MPV-to-NEU Ratio (MPVNR)	3,3 (0,93 - 9,38)	0,93 (0,93 - 9,38)	3,47 (1,28 - 7,26)
MPV-to-MON Ratio (MPVMR)	25,03 (9,19 - 53,31)	9,19 (9,19 - 52,7)	27,93 (11,18 - 53,31)
MPV-to-PLT Ratio (MPVPR)	0,42 (0,19 - 0,86)	0,2 (0,2 - 0,77)	0,42 (0,19 - 0,86)
NEU-to-LYM Ratio (NLR)	2,22 (0,56 - 9,07)	0,56 (0,56 - 9,07)	2,17 (0,88 - 6,09)
Derived NEU-to-LYM Ratio (dNLR)	1,53 (0,48 - 4,18)	0,48 (0,48 - 4,18)	1,55 (0,68 - 3,42)
NEU-to-LYM+PLT Ratio (N/LPR)	0,96 (0,23 - 4,29)	0,23 (0,23 - 4,29)	0,95 (0,35 - 2,65)
NEU-to-MON Ratio (NMR)	8,06 (3,63 - 17,8)	3,99 (3,99 - 13,5)	8,59 (3,63 - 17,8)
PDW-to-LYM Ratio (PDWLR)	11,03 (5,8 - 28,97)	5,8 (5,8 - 24,33)	11,33 (5,8 - 28,97)
PDW-to-NEU Ratio (PDWNR)	5,7 (1,56 - 17,51)	1,56 (1,56 - 16,87)	5,94 (2,16 - 17,51)
PDW-to-MON Ratio (PDWMR)	42,94 (15,32 - 105,04)	15,32 (15,32 - 82,09)	47,4 (19,08 - 105,04)
PLT-to-WBC Ratio (PWR)	46,45 (15,94 - 98,98)	22,33 (22,33 - 84,25)	49,98 (15,94 - 98,98)
PLT-to-LYM Ratio (PLR)	164,96 (63,25 - 634,34)	63,25 (63,25 - 413)	172,89 (68,28 - 634,34)
PLT-to-NEU Ratio (PNR)	82,36 (21,81 - 223,75)	29,09 (29,09 - 223,75)	88,63 (21,81 - 203,85)
PLT-to-MON Ratio (PMR)	637,49 (232,6 - 2103,73)	245,54 (245,54 - 1244,32)	727,62 (232,6 - 2103,73)
CRP-to-RDW Ratio (CRPRDWR)	0,21 (0,06 - 60,38)	0,06 (0,06 - 520,39)	1,07 (0,06 - 9,21)
ALP-to-RBC Ratio (ALPRBCR)	15,03 (7,2 - 66,31)	7,2 (7,2 - 66,31)	15,56 (7,35 - 66,31)
ALP-to-RDW Ratio (ALPRDWR)	4,75 (2,34 - 23,56)	2,62 (2,62 - 23,56)	4,64 (2,34 - 23,56)
ALP-to-PLT (ALPPR)	0,29 (0,13 - 1,86)	0,13 (0,13 - 1,86)	0,29 (0,14 - 1,86)
ALP-to-PDW Ratio (ALPPDWR)	4,22 (1,49 - 11,78)	1,76 (1,76 - 11,78)	4,23 (1,49 - 11,78)
LDH-to-RBC Ratio (LDHRBCR)	37,47 (24,12 - 119,44)	24,12 (24,12 - 119,44)	38,01 (25,61 - 55,66)
LDH-to-RDW Ratio (LDHRDWR)	11,77 (7,53 - 37,62)	7,65 (7,65 - 37,62)	11,23 (7,53 - 15,49)
LDH-to-LYM Ratio (LDHLR)	115,19 (46,36 - 520,39)	55,82 (55,82 - 520,39)	114,86 (46,36 - 472,14)
LDH-to-PLT Ratio (LDHPR)	0,73 (0,37 - 1,84)	0,4 (0,4 - 1,84)	0,69 (0,37 - 1,25)
LDH-to-PDW Ratio (LDHPDWR)	10,62 (4,34 - 36,87)	5,98 (5,98 - 36,87)	10,42 (4,34 - 19,24)
LDH-to-Albumin Ratio (LDHALBR)	3,71 (2,38 - 17,84)	2,38 (2,38 - 17,84)	3,49 (2,47 - 4,63)
NEU-to-HDL-c Ratio (NHR)	2,41 (0,56 - 9,81)	0,75 (0,75 - 9,81)	1,86 (0,56 - 5,27)
MON-to-HDL-c Ratio (MHR)	8 (1,44 - 25,77)	3,69 (3,69 - 25,77)	5,88 (1,44 - 13,32)
PLT-to-HDL-c Ratio (PHR)	166,94 (69,27 - 407,24)	74,29 (74,29 - 407,24)	138,13 (69,27 - 270,69)
Prognostic Nutritional Index (PNI)	453,15 (310,01 - 520,01)	310,01 (310,01 - 520,01)	453,62 (400,01 - 500,01)
CA 15.3-to-RBC Ratio (CA153RBCR)	4,7 (0,59 - 42,51)	0,61 (0,61 - 7,26)	6,36 (0,59 - 42,51)
CA 15.3-to-RDW Ratio (CA153RDWR)	1,34 (0,19 - 9,53)	0,26 (0,26 - 2,38)	1,67 (0,19 - 9,53)
CA 15.3-to-PLT Ratio (CA153PR)	0,08 (0,01 - 0,48)	0,01 (0,01 - 0,15)	0,1 (0,01 - 0,48)
CA 15.3-to-PLT Ratio (CA153PDWR)	1,39 (0,14 - 13,73)	0,14 (0,14 - 2,44)	1,89 (0,14 - 13,73)

Table 9. Inflammatory ratios —hematological, mixed and oncological—, displayed as the mean value of each parameter with the corresponding minimal (Min) and maximal (Max) value, for all patients and by gender: male and female.

RESULTS

This new approach to a routine blood and urine test —that is, including **Barcelona Critera** as part of the algorithm—, **has detected all cancer cases in the randomized controlled trial (RCT) validation set**, as can be seen in **Tables 10,12**.

In this way, in a sample size of 166 patients, **cancers detected were 14**: bladder (n = 1); breast (n = 5); colorectal (n = 1); liver (n = 1); lung (n = 2); melanoma (n = 1); prostate (n = 2); and suprarenal (n = 1).

Given that the prevalence of *Helicobacter pylori* infection in the validation set is very low (**Table 11**), it could be an indicative that the study population was biased. This is because the overall prevalence in Spain is much higher versus the one achieved in this study[105]. Since H. pylori infection is more frequent in people with lower income[106], this low prevalence in the validation group suggests that the study population is principally composed by middle-to-high class patients, with moderate-to-high income.

Variables/ Scenarios	RCT (Validation Set without BC) 95% CI	RCT (Validation Set with BC) 95% CI
Size	166	166
True Positive (TP)	14	14
False Positive (FP)	50	0
True Negative (TN)	102	152
False Negative (FN)	0	0

Table 10. Accuracy results obtained in the randomized controlled trial (RCT) validation set (n = 166 patients) without the inclusion of the Barcelona Criteria (BC) as part of the algorithm, compared with the results obtained with the same sample, but with the inclusion of these criteria..

Results without Barcelona Criteria —RCT (Validation Set without BC) 95% CI— were obtained according International Society of Oncology and Biomarkers (ISOBM) and European Group for Tumor Markers (EGTM) guidelines, as well as several pubications related to the use of TM in cancer diagnosis.

On the other side, results with Barcelona Criteria included were obtained by checking first for comorbidities that are well-known sources of elevation of TM without malignancy, resulting in FP.

Besides, no patient obtained a **Health Score** "B" or "A". **Health Score** is a scoring system developed over the course of the RCT as an aid to patient management, according the guidelines from the following organizations: the American Society of Hematology (ASH), the American Heart Association (AHA), the American Association of Clinical Endocrinology (AACE), the American Diabetes Association (ADA), the American Thyroid Association (ATA), the Bone Health and Osteoporosis Foundation (BHOF), the American Association for the Study of Liver Diseases (AASLD), the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI), the National Kidney Foundation (NKF), the American Urological Association (AUA), the American College of Obstetricians and Gynecologists (ACOG); and the American College of Rheumatology (ACR). The **Health Score** scale is: "E" for something found that needs to be checked urgently by a healthcare professional; "D" for something found that must be checked by a healthcare professional; "C" for something found that is recommended to be checked by a healthcare professional; "B" for something not relevant found —but, due to its nature no action is recommended, to avoid overdiagnosis—; and "A" for nothing found. In this way, all patients resulted with a **Health Score** C or lower (that is, "C", "D" or "E"), which means that all of them had at least one body function or system mild, moderate, severe or very severely altered, quite a few of them closely related with the comorbidities linked to the increase of TM by non-malignant causes, in accordance with the **Barcelona Critera**. In such manner, the number of patients with altered results, according to each function that was evaluated, were: hematologic system (n = 50); cardiovascular system (n = 78) —23 of them with a moderate-high 10-year risk of developing a Coronary Heart Disease (CHD)—; glucose metabolism (n = 39) —26 of them with a moderate-high 7.5-year risk for developing type 2 diabetes mellitus (DM2)—; pancreatic endocrine function (n = 9); thyroid function and hypothalamic-pituitary axis (n = 6); parathyroid function (n = 2); vitamin D function (n = 114) —12 of them with vitamin D deficiency and 102 with vitamin D insufficiency—; vitamin B12 function (n = 3); gastrointestinal tract (n = 65) —35 of them with an active H. pylori infection—; liver and bile-pancreatic exocrine function (n = 91) —67 of them with non-alcoholic fatty liver disease (NAFLD), 25 patients with non-alcoholic steatohepatitis (NASH) and 29 patients with a moderate 15-year risk for developing chronic liver disease (CLD)—; renal function (n = 29) —1 of them with a high 2-year risk for kidney failure (KF)—; uric acid metabolism (n = 2); prostate function (n = 7); and inflammatory response (n = 4).

As mentioned above, serum TM levels can be increased in several non-malignant conditions —mainly those affecting the liver and the kidneys, among others (**Tables 1 and 2**)—. This means **TM's upper reference levels (URL) will depend on the patient's condition so, any test using TM but not checking also for other comorbidities, could give a very high rate of FP**.

In this sense, the interpolation of the learnings about the impact of **non-alcoholic fatty liver disease (NAFLD)**, **non-alcoholic steatohepatitis (NASH)**, **liver fibrosis** or **liver cirrhosis** on several tumor markers (e.g. AFP, CA 15.3, CA 19.9, CA 72.4, CA 125, CEA, CYFRA 21-1, HE4, NSE, ProGRP, PSA and SCC), is a very useful method to discard FP due to liver disease, since **these TM can be increased in a moderate, high or very high way without cancer**. In the same way, the **estimated glomerular filtration rate (eGFR)** —from the **Kidney Disease Improving Global Outcomes (KDIGO) guidelines**—, and the **urine albumin-to-creatinine ratio (ACR)**, could increase serum levels of AFP, CA 15.3, CA 19.9, CA 72.4, CA 125, CEA, CYFRA 21-1, NSE, ProGRP and PSA, as kidney disease affect these TM. Nevertheless, **in the case of kidney failure (KF), HE4, SCC and S100 can not be taken into account**, because these TM levels can be higher than malignant ones.

The application of all these learnings translates into an **overall increase of the algorithm's performance if compared with the one developed with the training set. This is specially relevant in the values of sensitivity and negative predictive value (NPV), which are the most important ones in cancer screening, because they depend on FP** —reduction in FP translates into an increase of sensitivity and NPV—.

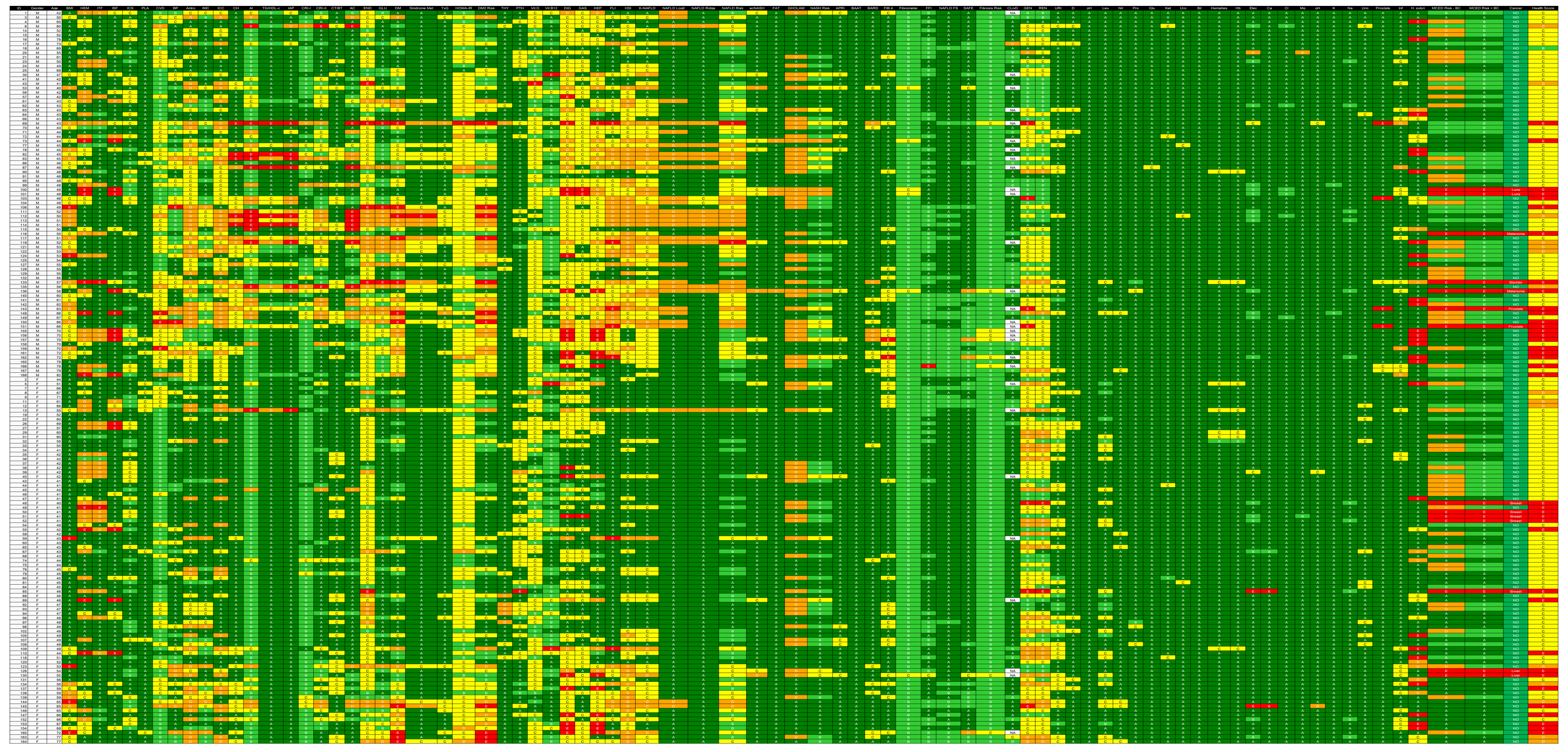


Table 11. Complete results for all patients in the validation set, being each line one patient. Columns correspond to the following data: clinical data of the patients, a series of body functions and systems, cancer risk without and with the application of the Barcelona Criteria —to evaluate its efficiency—, cancer type, and the Health Score. Different benign comorbidities are determined by the assessment of many functions (e.g., hepatic —with independent assessment to jaundice—, pancreatic exocrine, renal, and prostate), that can cause FP results of TM. Each box is colored according to the result obtained, which is scored from "E" to "A", meaning "E" that the result is highly concerning and requires medical consultation; meaning "D" that the result is very concerning; meaning "C" that the result is concerning; meaning "B" that the result is no concerning; and meaning "A" that the result is normal. The column on the far right corresponds to the overall Health Score, and refers to the overall results obtained. It can be observed that no one reached a score higher than "C" —no one achieved a "B or "A" score—, meaning that in every patient, an abnormality of some physiological function was found.

CONCLUSIONS

- This innovative **non-invasive blood and urine-based biomarker algorithm holds promise in providing timely and accurate screening of multiple cancer —even in early stages—**, before symptoms and signs appear and **when treatment is most likely to be successful. It is particularly targeted to individuals aged 40 years and above**. In this way, it is able to **detect up to 42 different solid tumors**, directly identifying the following list of 16: **bladder cancer (BLC), breast cancer (BC), cervical cancer (CC), colorectal cancer (CRC), gastric cancer (GC), head & neck cancer (HNC), kidney cancer (KC), liver cancer (LCa), lung cancer (LC), melanoma, ovarian cancer (OC), pancreatic cancer (PC), prostate cancer (PCa), testicular cancer (TCa), thyroid cancer (TC), and parathyroid cancer (PTHC); and 5 hematological malignancies: leukemias, Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), myelodysplastic syndrome (MDS), and primary myelofibrosis (PMF)**.
- This algorithm also has the **ability to categorize several cancer types: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC) —for LC—; hepatocellular carcinoma (HCC), cholangiocarcinoma (CC) —also known as bile duct cancer (BDC)—, angiosarcoma (AS), hemangiosarcoma (HSA), and hepatoblastoma (HB) —for LCa—; germ cell tumors (GTC), sex cords-stromal tumors (SCST), or epithelial tumors —for OC—. Moreover, it also identifies a few cancer subtypes: adenocarcinoma or squamous cell carcinoma (SCC) —for NSCLC—; and serous and mucinous carcinoma —for ovarian epithelial tumors—**. However, to continue enhancing the algorithm's accuracy in the general population, further exploration would be needed. A **multicenter, international clinical trial of 26,000 patients** will be conducted from March 2025 until December 2026. In this way, additional analytes will be also added —like carbohydrate antigen 62 (CA-62), a promising new tumor marker for epithelial carcinomas—.
- The **use of the Barcelona Criteria (BC) —or any other method to avoid FP—, should be a must in any procedure that implies the use of TM, since non-malignant diseases can also increase TM levels**. This study demonstrates how important their inclusion is to **improve cancer detection and avoid overdiagnosis, by eliminating FP** as much as possible. In this sense, **any test using TM but not checking also for other comorbidities, could give a very high rate of FP**. The inclusion of the BC implies, at least, the addition to the rest of TM (**Table 1** and **Table 2**), a hepatic panel —hemogram, albumin, total protein, ALP, AST, ALT, GGT, LDH, total cholesterol, HDL-c, non-HDL-c, LDL-c, VLDL-c, triglycerides, total bilirubin, direct bilirubin, glucose, and HbA1c—, as well as a renal panel —serum electrolytes, albumin, glucose, serum creatinine, urine albumin, and urine creatinine—, among others laboratory determinations. This is because TM are normally catabolyzed in the liver and excreted through the kidneys, so any disease affecting any of these two organs could be an important source of FP in TM. Moreover, BC are also useful to discard any FP result caused by technical interferences —hemolyzed, lipemic, or icteric samples, among others—, that affect the results of TM —e.g., an elevated consumption of biotin[107,108] (vitamin B7) without medical prescription to strengthen hair and nails, produces the well-known "hook effect", which affects the results by saturating the testing antibodies—.
- Despite the fact that results obtained in the algorithm modeling —training set— were very high (Se = 0.95, Sp = 0.73), and results obtained in this RCT —algorithm validation set— were even higher (Se = 1.00, Sp = 1.00), it can not be ignored that, firstly, the **analysis performed for the algorithm modeling was performed by different suppliers as well different techniques —consequently, there may be variability and bias—**; and, secondly, the **RCT's sample size was very low (n = 166)**.
- The panels included for FP avoidance, not only allow the detection of up to 47 cancers —even in early stages—, but **also allow the detection of main conditions that, whitin years, can promote cancer to appear —specially relevant for those diseases related to overweight, obesity and metabolic syndrome (MetS), as they are closely linked to cancer causes and also are considered cancer risk factors—, offering to healthcare professionals a very reliable and valuable tool to take corrective measures**.
- The case study explained demonstrates how **early detection is key in the diagnosis of cancer, reducing lead time and potentially improving 5-YRSR, prognosis and treatment**.
- On the other hand, the case study also underlines the need to encourage healthcare professionals to explore **how Evidence-Based Laboratory Medicine (EBLM)**[109,110], new technologies —mainly **machine learning (ML) algorithms**—, and the learnings obtained from the **Barcelona Criteria** can help them to **improve diagnosis accuracy, reduce medical errors and misdiagnoses, as well as avoiding invasive procedures**.
- The most critical aspect of achieving the goals of the RCT was the management of the follow-up visits. This is because **the no assistance to any of these visits implies losing a patient, as well as the cost of the performed analytics**. Therefore, this is specially relevant in the upcoming RCT, where the total cost of the study will be of USD 18 M, **being USD 3 M exclusively dedicated for analytics** and USD 7 M for the contract research organization (CRO) —the two largest budget items related to patients—.
- Finally, **despite promising results have been achieved, there is still a lot of information to be obtained from blood**, and a lot of work to be done to understand it.

Variables/ Scenarios	Algorithm Modeling (Training Set) 95% CI	RCT (Validation Set without BC) 95% CI	RCT (Validation Set with BC) 95% CI
Sample Size (n)	192,090	166	166
Sensitivity	0.95	1.00	1.00
Specificity	0.73	0.67	1.00
AUROC	0.92	0.68	1.00
PPV	0.77	0.21	1.00
NPV	0.93	1.00	1.00

Table 12. Sensitivity (Se), specificity (Sp), AUROC, positive predictive value (PPV) and negative predictive value (NPV) results obtained in the randomized controlled trial (RCT) validation set (n = 166 patients) without the Barcelona Criteria (BC) included as part of the algorithm, compared with the results obtained with the same sample, but with these critera included. It can be seen how the Barcelona Criteria increase all the accuracy parameters, especially the Sp, that reaches a 1.00 from the 0.67, as well as the PPV, that reaches a 1.00 from 0.21 —the main weak points of TM[111–116]—.

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References:
1. The International Agency for Research on Cancer (IARC). (s. f.). Global Cancer Observatory. https://gco.iarc.fr/en
2. Ryan, D., Barquera, S., Barata Cavalcanti, O., Ralston, J. (2021). The Global Pandemic of Overweight and Obesity. In: Kickbusch, I., Ganten, D., Moeti, M. (eds) Handbook of Global Health. Springer, Cham
3. Pati S, Irfan W, Jameel A, Ahmed S, Shahid RK. Obesity and Cancer: A Current Overview of Epidemiology, Pathogenesis, Outcomes, and Management. Cancers (Basel). 2023 Jan 12;15(2):485
4. Underferth, D. (2020, 8 mayo). How does obesity cause cancer? MD Anderson Cancer Center. https://www.mdanderson.org/publications/focused-on-health/how-does-obesity-cause-cancer.h27Z1591413.html
5. FastStats. (n.d.). Leading Causes of Death. https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm
6. Coldtiz G A, et al. Epidemiology — identifying the causes and preventability of cancer? Nature Reviews. 2006,5,75-8
7. The International Agency for Research on Cancer (IARC). (s. f.). Global Cancer Observatory (Data of the 5-YRSR)
8. Cancer blood tests: Lab tests used in cancer diagnosis. (2022, March 10). Mayo Clinic. https://www.mayoclinic.org/diseases-conditions/cancer/in-depth/cancer-diagnosis/art-20046459
9. Molina R., et al. Tumor markers: theory or reality?. Med Clin (Barc). 1994;102:189-95
10. Breast Imaging Reporting & Data System. (n.d.). American College of Radiology. https://www.acr.org/Clinical-Resources/Reporting-and-Data-Systems/Bi-Rads
11. Trapé J., et al. Factors influencing blood tumor marker concentrations in the absence of neoplasia. Tumor Biology. 2024,46:35- 63
12. American Association for Cancer Research (AACR). (2024, September 10). American Association for Cancer Research (AACR). https://www.aacr.org/
13. Ministerio de Sanidad, Consumo y Bienestar Social - Statical Site of the NHS - Spanish National Health Survey. (n.d.). https://www.sanidad.gob.es/en/estadEstudios/estadisticas/encuestaNacional/home.htm
14. Marcus PM. Assessment of cancer screening: a primer [Internet]. Bethesda (MD): National Cancer Institute (US); 2019 Nov. Chapter 2, Behind the scenes. Available from: https://www.ncbi.nlm.nih.gov/books/NBK550219/
15. Calleja S. J. et al., Diagnostic accuracy of the CxPREVENTIVE breast model for early detection of breast cancer.. JCO 40, e13578-e13578(2022)
16. Calleja S. J. et al., Diagnostic accuracy of the CxPREVENTIVE Colon model for early detection of colorectal cancer.. JCO 40, e15518-e15518(2022)
17. Santotoribio, J.D. et al. An innovative evidence-based laboratory medicine (EBLM) test to help doctors in the screening of hepatocellular carcinoma (HCC). Annals of Oncology, Volume 35, S90
18. Santotoribio, J.D. et al. An innovative evidence-based laboratory medicine (EBLM) test to help doctors in the screening of ovarian cancer. ESMO Open, Volume 9, 103506
19. Calleja S. J. et al., An Innovative Evidence-Based Laboratory Medicine (EBLM) Test to Help Doctors in the Assessment of Lung Cancer. IASLC WCLC 2024, Poster EP.04D.02
20. Molina R, et al. Marcadores tumorales: estado actual y perspec- tivas de futuro II. Barcelona: Roche Diagnostic S.L.; 2003.
21. Molina R, et al. S-100 protein serum levels in patients with benign and malignant diseases: false-positive results related to liver and renal function. Tumour Biol. 2002;23:39-44. doi: 10.1159/000048687
22. Filella X, et al. Prostate specific antigen in non-serum samples from women. Int J Biol Markers. 1995;10:238-9. doi: 10.1177/172460089501000410
23. Trapé J, et al. Rigo-BonninR.Oncology Section of the Catalan Association of Clinical Laboratory Science. Increased plasma concentrations of tumour markers in the absence of neoplasia. Clin Chem Lab Med. 2011;49:1605-20
24. Filella X, et al. Tumor markers in patients with chronic renal failure. Int J Biol Markers. 1990;5:85-8
25. Trape´J, et al.Reference change value for alpha-fetoprotein and its application in early detection of hepatocellular carcinoma in patients with hepatic disease. Clin Chem. 2003;49:1209- 11
26. Sturgeon CM, et al. National Academy of Clinical Biochemistry. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Clin Chem. 2008;54:e11-79
27. Molina R, et al. Utility of serum tumor markers as an aid in the differential diagnosis of patients with clinical suspicion of cancer and in patients with cancer of unknown primary site. Tumour Biol. 2012;33:463-74
28. Bosch X, et al. Tumour markers with clinically controlled cut-offs for suspected cancer. Eur J Clin Invest. 2021;51:e13523
29. Molina R, et al. S-100 protein serum levels in patients with benign and malignant diseases: false-positive results related to liver and renal function. Tumour Biol. 2002;23:39-44
30. Danisman A, et al. Do renal failure and hemodialy- sis have any effect on the elimination of free and total prostate-specific antigen? Eur Urol. 2000;37:579-81
31. Collazos J, et al. Neuron-specific enolase concentrations in serum in benign liver diseases. Clin Chem. 1991;37:579-81
32. Collazos J, et al. Measurement of the serum tumor marker neuron-specific enolase in patients with benign pulmonary diseases. Am J Respir Crit Care Med. 1994;150:143-5
33. Collazos J, et al. Evaluation of the behavior of carcinoembryonic antigen in cirrhotic patients. Int J Biol Markers. 1992;7(4):244-8
34. Cure MC, et al. Requests for tumor marker tests in Turkey without indi- cations and frequency of elevation in benign conditions. Asian Pac J Cancer Prev. 2012;13:6485-9
35. Coppolino G, et al. Tumour markers and kidney function: a systematic review. Biomed Res Int. 2014;2014:647541.
36. Purut YE, et al. Effect of the coronavirus pandemic on tumor markers. J Med Virol. 2021;93:5405-8
37. Sarandakou A, et al.Tumor markers in biological fluids associated with pregnancy. CritRevClin Lab Sci. 2007;44:151-78
38. Nwabuobi C, et al. hCG: Biological Functions and Clinical Applications. Int J Mol Sci. 2017;18:2037
39. Meznerics FA, et al. Multibiomarker disease activity score: an objective tool for monitoring rheumatoid arthritis? A systematic review and meta-analysis, Rheumatology;62:6, June 2023:2048-2059
40. Chin CA, et al. Chapter 16 - Evidence-based laboratory medicine. Contemporary Practice in Clinical Chemistry (Fourth Edition), 2020:265-288
41. Ghadage, O. P. (2023, February 20). What is Recursive Algorithm? Types and Method. Simplilearn.com. https://www.simplilearn.com/tutorials/data-structure-tutorial/recursive-algorithm
42. Mahdavi-Roshan M, Shoaibinobarian N, Noormohammadi M, Fakhr Mousavi A, Savar Rakhsh A, Salari A, Ghorbani Z. Inflammatory Markers and Atherogenic Coefficient: Early Markers of Metabolic Syndrome. Int J Endocrinol Metab. 2022 Aug 28;20(4):e127445.
43. Çelik E, et al. The Effect of Untraditional Lipid Parameters in the Development of Coronary Artery Disease: Atherogenic Index of Plasma, Atherogenic Coefficient and Lipoprotein Combined Index. J Saudi Heart Assoc. 2021 Sep 20;33(3):244-250
44. Dobiásová M, Frohlich J, Sedová M, Cheung MC, Brown BG. Cholesterol esterification and atherogenic index of plasma correlate with lipoprotein size and findings on coronary angiography. J Lipid Res. 2011 Mar;52(3):566-71. doi: 10.1194/jlr.P011668. Epub 2011 Jan 11.
45. Lumu W, et al. Atherogenic index of plasma and its cardiovascular risk factor correlates among patients with type 2 diabetes in Uganda. Afr Health Sci. 2023 Mar;23(1):515-527
46. Castelli WP, et al. Summary estimates of cholesterol used to predict coronary heart disease. Circulation. 1983 Apr;67(4):730-4. doi: 10.1161/01.cir.67.4.730
47. Schwertner HA, et al. Comparison of various lipid, lipoprotein, and bilirubin combinations as risk factors for predicting coronary artery disease. Atherosclerosis. 2000 Jun;150(2):381-7
48. Senthilkumari S, et al. Is Lipid Tetrad Index a Promising Atherogenic Index in Acute Coronary Syndrome?. International Journal of Scientific Study, 2016;4:8
49. Morais CA, et al. Índices Lipídicos Tetravalente (LTI) e Pentavalente (LPI) em indivíduos saudáveis [Lipid Tetrad Index (LTI) and Lipid Pentad Index (LPI) in healthy subjects]. Arq Bras Cardiol. 2013 Apr;100(4):322-7
50. Das B, et al. Lipid Pentad Index: A novel bioindex for evaluation of lipid risk factors for atherosclerosis in young adolescents and children of premature coronary artery disease patients in India. Clin Biochem. 2007 Jan;40(1-2):18-24
51. Saif-Ali R, et al. Relationship of metabolic syndrome defined by IDF or revised NCEP ATP III with glycemic control among Malaysians with Type 2 Diabetes. Diabetol Metab Syndr 12, 67 (2020)
52. Liao Y, et al. Critical evaluation of adult treatment panel III criteria in identifying insulin resistance with dyslipidemia. Diabetes Care. 2004 Apr;27(4):978-83
53. Unger G, et al. Triglycerides and glucose index: a useful indicator of insulin resistance. Endocrinol Nutr. 2014 Dec;61(10):533-40
54. Vladu IM, et al. Insulin resistance quantified by the value of HOMA-IR and cardiovascular risk in patients with type 2 diabetes. Exp Ther Med. 2022 Jan;23(1):73
55. Paracha AI, et al. Diagnostic Accuracy of Markers of Insulin Resistance (HOMA-IR) and Insulin Sensitivity (QUICKI) in Gestational Diabetes. J Coll Physicians Surg Pak. 2021 Sep;31(9):1015-1019
56. Felton JL, et al. HOMA2-B enhances assessment of type 1 diabetes risk among TrialNet Pathway to Prevention participants. Diabetologia. 2022 Jan;65(1):88-100
57. Huang X, et al. Validation of the Fatty Liver Index for Nonalcoholic Fatty Liver Disease in Middle-Aged and Elderly Chinese. Medicine (Baltimore). 2015 Oct;94(40):e1682
58. Kahl S, et al. Comparison of liver fat indices for the diagnosis of hepatic steatosis and insulin resistance. PLoS One. 2014 Apr 14;9(4):e94059
59. Lee JH, et al. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis. 2010 Jul;42(7):503-8
60. Jeong S, et al. Development of a simple nonalcoholic fatty liver disease scoring system indicative of metabolic risks and insulin resistance. Ann Transl Med. 2020 Nov;8(21):1414
61. Yip TC, et al. Laboratory parameter-based machine learning model for excluding non-alcoholic fatty liver disease (NAFLD) in the general population. Aliment Pharmacol Ther. 2017 Aug;46(4):447-456
62. Wang J, et al. Diagnostic value of alcoholic liver disease (ALD)/nonalcoholic fatty liver disease (NAFLD) index combined with gamma-glutamyl transferase in differentiating ALD and NAFLD. Korean J Intern Med. 2016 May;31(3):479-87
63. Wu XX, et al. acNASH index to diagnose nonalcoholic steatohepatitis: a prospective derivation and global validation study. EClinicalMedicine. 2021 Oct 1;41:101145
64. Varghese, J., et al. FAT score: an Indian insight to a novel diagnostic score to differentiate non-alcoholic steatohepatitis (NASH) from simple steatosis. Egypt J Intern Med 33, 42 (2021)
65. Gholam PM, et al. Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol. 2007 Feb;102(2):399-408
66. Bril F, et al. Performance of Plasma Biomarkers and Diagnostic Panels for Nonalcoholic Steatohepatitis and Advanced Fibrosis in Patients With Type 2 Diabetes. Diabetes Care. 2020 Feb;43(2):290-297
67. Lin ZH, et al. Performance of the aspartate aminotransferase-to-platelet ratio index for the staging of hepatitis C-related fibrosis: an updated meta-analysis. Hepatology. 2011 Mar;53(3):726-36
68. Hernandez Roman J, et al. The role of noninvasive biomarkers in diagnosis and risk stratification in nonalcoholic fatty liver disease. Endocrinol Diabetes Metab. 2020 Apr 5;3(4):e00127
69. Cichoż-Lach H, et al. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. Med Sci Monit. 2012 Dec;18(12):CR735-40
70. Xu XL, et al. The role of fibrosis index FIB-4 in predicting liver fibrosis stage and clinical prognosis: A diagnostic or screening tool? J Formos Med Assoc. 2022 Feb;121(2):454-466
71. Van Dijk A-M, et al. Systematic Review with Meta-Analyses: Diagnostic Accuracy of FibroMeter Tests in Patients with Non-Alcoholic Fatty Liver Disease. Journal of Clinical Medicine. 2021; 10(13):2910
72. Forns, Xavier, et al. Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology;36
73. Angulo P, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007 Apr;45(4):846-54
74. Sripongpun P, et al. The steatosis-associated fibrosis estimator (SAFE) score: A tool to detect low-risk NAFLD in primary care. Hepatology. 2023 Jan 1;77(1):256-267
75. Ortega-Alonso A, et al. Clinical Features and Risk Factors in Drug-Induced Liver Injury. International Journal of Molecular Sciences. 2016; 17(5):714
76. Åberg F, et al. Development and validation of a model to predict incident chronic liver disease in the general population: The CLivD score. Journal of Hepatology;77(2), 2022: 302-311
77. Said NM, et al. Three gold indicators for breast cancer prognosis: a case-control study with ROC analysis for novel ratios related to CBC with (ALP and LDH). Mol Biol Rep. 2019 Apr;46(2):2013-2027
78. Wu F, et al. Prognostic value of baseline hemoglobin-to-red blood cell distribution width ratio in small cell lung cancer: A retrospective analysis. Thorac Cancer. 2020 Apr;11(4):888-897
79. Szkandera J, et al. The lymphocyte/monocyte ratio predicts poor clinical outcome and improves the predictive accuracy in patients with soft tissue sarcomas. Int J Cancer. 2014 Jul 15;135(2):362-70
80. Huszno J, Kolosza Z. Prognostic value of the neutrophil-lymphocyte, platelet-lymphocyte and monocyte-lymphocyte ratio in breast cancer patients. Oncol Lett. 2019 Dec;18(6):6275-6283
81. Cao J, et al. Role of combined use of mean platelet volume-to-lymphocyte ratio and monocyte to high-density lipoprotein cholesterol ratio in predicting patients with acute myocardial infarction. J Cardiothorac Surg. 2023 May 6;18(1):172
82. Shang QX, et al. Clinical and prognostic significance of preoperative lymphocyte-monocyte ratio, neutrophil-lymphocyte ratio and neutrophil-monocyte ratio on esophageal squamous cell carcinoma patients. Transl Cancer Res. 2020 Jun;9(6):3903-3914
83. Dirican A, et al. Do the derived neutrophil to lymphocyte ratio and the neutrophil to lymphocyte ratio predict prognosis in breast cancer? Int J Clin Oncol. 2015 Feb;20(1):70-81
84. Gameiro J, et al. Neutrophil, lymphocyte and platelet ratio as a predictor of mortality in septic-acute kidney injury patients, Nefrología;40:4, 2020: 461-468
85. Tang F, et al. The Neutrophil-to-Monocyte Ratio and Platelet-to-White Blood Cell Ratio Represent Novel Prognostic Markers in Patients with Pancreatic Cancer. Gastroenterol Res Pract. 2021 May 24;2021:6693028
86. Köse A, et al. The Usefulness of Platelet Distribution Width and Platelet Distribution Width to Lymphocyte Ratio in Predicting Severity and Outcomes in Patients with Snakebite. Wilderness Environ Med. 2021 Sep;32(3):284-292
87. Wang L, et al. The relationship between the platelet to leukocyte ratio and mechanical thrombectomy outcomes in acute ischemic stroke patients. Neurol Res. 2020 Oct;42(10):890-896
88. Zhai G, et al. Platelet-lymphocyte ratio as a new predictor of in-hospital mortality in cardiac intensive care unit patients. Sci Rep. 2021 Dec 8;11(1):23578
89. Jin P, et al. Platelet-to-neutrophil ratio is a prognostic marker for 90-days outcome in acute ischemic stroke. J Clin Neurosci. 2019 May;63:110-115
90. Zhou J, et al. Platelet-to-Monocyte Ratio as a Novel Promising Agent for the Prognosis of Hepatitis B Virus-Associated Decompensated Cirrhosis. Can J Gastroenterol Hepatol. 2023 Jul 14;2023:6646156
91. Wei XB, et al. Combined efficacy of C-reactive protein and red blood cell distribution width in prognosis of patients with culture-negative infective endocarditis. Oncotarget. 2017 Apr 6;8(41):71173-71180
92. Zhang Y, et al. Preoperative alkaline phosphatase-to-platelet count ratio as a prognostic factor for hepatocellular carcinoma with microvascular invasion. Cancer Med. 2023 Sep;12(17):17545-17558
93. Avcioglu G, et al. The importance of LDH/Albumin, LDH/Lymphocyte, and LDH/Platelet ratios in the evaluation of COVID-19 B.1.1.7 variant. Turkish Journal of Biochemistry, vol. 47, no. 5, 2022, pp. 656-664
94. Pan X, et al. Association of Neutrophil to High-Density Lipoprotein Cholesterol Ratio with Cardiac Ultrasound Parameters and Cardiovascular Risk: A Cross-Sectional Study Based on Healthy Populations. J Inflamm Res. 2023 Apr 27;16:1853-1865
95. Miao T, et al. Monocyte-to-High-Density Lipoprotein-Cholesterol Ratio Predicts Prognosis of Hepatocellular Carcinoma in Patients with Metabolic-Associated Fatty Liver Disease. J Hepatocell Carcinoma. 2024 Jan 18;11:145-157
96. Lu CF, et al. Association between the platelet/high-density lipoprotein cholesterol ratio and nonalcoholic fatty liver disease: results from NHANES 2017-2020. Lipids Health Dis. 2023 Aug 11;22(1):130
97. Liu, M, et al. Prognostic Nutritional Index (PNI) as a potential predictor and intervention target for perioperative ischemic stroke: a retrospective cohort study. BMC Anesthesiol 23, 268 (2023)
98. Steele T, et al. Assessment and clinical course of hypocalcemia in critical illness. Crit Care 17, R106 (2013)
99. Madeo B, et al. Serum Calcium to Phosphorous (Ca/P) Ratio Is a Simple, Inexpensive, and Accurate Tool in the Diagnosis of Primary Hyperparathyroidism. JBMR Plus. 2017 Nov 2;2(2):109-117
100. Salman E, et al. Investigation of the urinary sodium-to-potassium ratio target level based on the recommended dietary intake goals for the Japanese population: The INTERMAP Japan. Hypertens Res 45, 1850-1860 (2022)
101. Luke DR, et al. Validity of creatinine clearance estimates in the assessment of renal function. Clin Pharmacol Ther. 1990 Nov;48(5):503-8
102. Stevens LA, Zhang Y, Schmid CH. Evaluating the performance of equations for estimating glomerular filtration rate. J Nephrol. 2008 Nov-Dec;21(6):797-807
103. Uchino S, et al. The meaning of the blood urea nitrogen/creatinine ratio in acute kidney injury, Clinical Kidney Journal;5:2. April 2012:187-191
104. Wang J, et al. High Normal Urinary Albumin-Creatinine Ratio Is Associated With Hypertension, Type 2 Diabetes Mellitus, HTN With T2DM, Dyslipidemia, and Cardiovascular Diseases in the Chinese Population: A Report From the REACTION Study. Front Endocrinol (Lausanne). 2022 May 20;13:864562
105. Martín de Argila C, et al. Helicobacter pylori y enfermedades relacionadas. Servicio de Gastroenterología. Hospital Ramón y Cajal 2004,3:6,262-267
106. Yuan C, et al. The global prevalence of and factors associated with Helicobacter pylori infection in children: a systematic review and meta-analysis. The Lancet Child & Adolescent Health,6:3, 2022:185-194
107. Ohn J. J., et al. Assessment of biotin supplementation among patients in an outpatient dermatology clinic. J Am Acad Dermatol 2019;81:620-1
108. Ghazal K., et al. Thormone Immunoassay Interference: A 2021 Update. Ann Lab Med. 2022 Jan 1;42(1):3-23
109. Badrick T. Evidence-based laboratory medicine. Clin Biochem Rev. 2013 Aug;34(2):43-6
110. Plebani M. Quality in laboratory medicine: an unfinished journey. J Lab Precis Med 2017;2:63
111. Mizuno, T. , Goto, T. , Shimojo, K. , Watanabe, N. and Tanaka, T. (2021) Clinical Utility of Tumor Markers. Open Journal of Pathology, 11, 38-57
112. Filella Xavier, et al. Clinical usefulness of circulating tumor markers. Clin. Chem. and Laboratory Medicine (CCLM), vol. 61, no. 5, 2023, pp. 895-905
113. Mahadevarao Premnath S, Zubair M. Laboratory Evaluation of Tumor Biomarkers. [Updated 2023 Oct 28]
114. Appropriate use of tumour markers - bpacnz. (n.d.). https://bpac.org.nz/2023/tumour-markers.aspx
115. Perkins GL, et al. Serum tumor markers. Am Fam Physician. 2003 Sep 15;68(6):1075-82
116. Duffy MJ. Tumor markers in clinical practice: a review focusing on common solid cancers. Med Princ Pract. 2013;22(1):4-11. doi: 10.1159/000338393. Epub 2012 May 15

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