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WJGP mainly publishes articles reporting research results and findings obtained in the field of gastrointestinal pathophysiology and covering a wide range of topics including disorders of the esophagus, stomach and duodenum, small intestines, pancreas, biliary system, and liver.

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EVIDENCE REVIEW

Pancreatic neuroendocrine tumors: A case-based evidence review

Naveena Rikhraj, Cornelius J Fernandez, Vanishri Ganakumar, Joseph M Pappachan

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Abstract

Pancreatic neuroendocrine tumors (pNETs) are rare, presenting significant challenges in timely diagnosis and subsequent treatment. The clinical and pathobiological behavior of these tumors varies significantly, making follow-up and therapeutic approaches challenging for clinicians. Although the majority of these neoplasms are hormonally inactive, some can be associated with endocrine dysfunction. Very rarely, a nonfunctional tumor can later become hormonally active, further complicating prognostication and management. Depending on the character of the disease, clinical picture and prognosis, different treatment modalities are instituted with varying effectivities. We recently came across a unique case of nonfunctioning malignant pNET at an advanced stage, metastatic disease upon diagnosis, managed medically with somatostatin analog therapy (Octreotide) and targeted therapy (Everolimus) with stable disease for 40 months that subsequently turned out to become functional (insulinoma). With the aid of this unique case, we update the current clinical, diagnostic and therapeutic approach to pNETs in this evidence-based review.

Key Words: Pancreatic neuroendocrine tumour; Somatostatin analog therapy; Insulinoma; Nonfunctional pNET; Metastatic disease; Somatostatin receptor imaging

Core Tip: Pancreatic neuroendocrine tumors (pNETs) are rare neoplasms with variable clinical and pathobiological characteristics posing diagnostic and therapeutic challenges. Mostly nonfunctional with low growth rates, some cases can be functional while others can run an aggressive clinical course. Hormonal, imaging and histological assessments are essential for planning appropriate diagnosis, prognosis and management. Therapeutic strategies depend on the tumor grade, functionality and presence of metastatic disease. Nonfunctioning pNETs very rarely turn functional during the advanced disease course. Presenting a unique nonfunctioning metastatic pNET case, later evolving to an insulinoma, we update the diagnostic, prognostic and management strategies for pNETs in the evidence-based review.

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INTRODUCTION

Epidemiology of pancreatic neuroendocrine tumors

Neuroendocrine neoplasms (NENs) refer to neoplasms originating from the enterochromaffin cells of the embryonic gut exhibiting both neural and endocrine differentiation. While the majority of NENs are well-differentiated, less aggressive, but potentially malignant NETs (neuroendocrine tumors), the remaining are poorly differentiated and aggressive NECs (neuroendocrine carcinomas). As per the latest classification, NENs can be divided into gastroenteropancreatic NENs (GEP-NENs) and lung NENs[1]. GEP-NENs constitute 55%-70% of all NENs, 12%-20% of which are formed by pancreatic NENs (pNENs)[2].

The pNENs account for only 1%-2% of all pancreatic neoplasms, and pancreatic NETs (pNETs) account for nearly 90% of all pNENs[3]. The pNENs are mostly diagnosed in the sixth decade of life, and they exhibit a minimal male preponderance. Nearly 70% of the pNENs are non-functioning, whereas 30% are functioning, secreting hormones or peptides associated with the clinical syndrome[4]. Those tumors with detectable peptides on biopsy specimens but that do not exhibit features of hormone hypersecretion syndrome are still considered non-functioning. While most pNENs are sporadic, nearly 10% are associated with hereditary syndromes, including Multiple Endocrine Neoplasia type 1 or type 4 (MEN1 or MEN4), von Hippel-Lindau syndrome, neurofibromatosis type 1 and tuberous sclerosis. The hereditary pNENs are often multifocal, well-differentiated, functioning, possibly secreting multiple peptide hormones simultaneously, with a young age of onset, and are associated with ectopic production of hormones and accompanied by other endocrine disorders or malignancies. Though pNENs are less aggressive with better 5-year survival rates compared to pancreatic adenocarcinomas, they are associated with nearly 23.1% recurrence rates within 8.1 years, even after curative resection[5].

A population-based study using surveillance epidemiology and end results (SEER) 18 registry data showed that in comparison to 2000-2008, the annual incidence of pNETs has increased from 0.27 per 100000 to 1.00 per 100000 in the study period 2009-2016[6]. This increase in annual incidence is accompanied by the early detection of particularly the asymptomatic non-functioning NETs in their early stages, thereby resulting in an improvement in median overall survival from 46 months to 85 months. Moreover, the improved median overall survival was consistent over different stages of the disease - localized disease at 83%, regional disease at 67%, and metastatic disease at 28%[6].

The overall incidence of GEP-NECs is rare, but several studies report increasing incidence particularly across North America, Asia and Europe[7,8]. The SEER 18 registry reported an incidence rate of 9.36 per 100000, nearly 90% of which are lung NECs (8.36 per 100000, 95% small cell NECs and 5% non-small cell NECs), whereas pNECs constitute only 0.07 per 100000 (small cell NECs nearly 50%)[9]. The pNECs have one of the shortest median survivals at 5.7 months [multivariate hazard ratio (HR): 1.10; 95% confidence interval (CI): 1.03-1.18, $P < 0.001$]. This could be attributed to the advanced stage at diagnosis (75.6% of the pNEC patients presenting with distant metastasis) in comparison to other GEP-NEC subtypes[9].

Due to this cumulative disease burden, studying the pathophysiology, clinical presentation, and response to treatment of pNETs and pNECs make it interesting and relevant.

Etiopathogenesis of pNETs

The pNETs are derived from primitive endoderm, which differentiate into several gastrointestinal (GI) epithelial lineages, inclusive of enterochromaffin cells. These enterochromaffin cells are categorised based on morphology, location and expression of peptide hormones. The proliferation of these cells is dependent on various transcription factors, for example, Neurogenin 3 in the pancreas, which delineates the Notch signalling pathway responsible for secretory and absorptive cell lineages[10].

Germline mutations in tumour suppressor genes *MEN1, VHL, NF1,* and *TSC1/2* account for 10% of familial syndrome-derived pNETs. The majority of pNETs are sporadic, resulting from somatic cell mutations for proteins involved in chromatin modelling, for example, *MEN1, DAXX* and *ATRX*. Encoding protein mutations in the mTOR pathway have shown a correlation to the formation of pNETs[11]. Alongside the presence of serum biomarkers, these have vital implications for targeted therapy. NECs can present with specific genetic alterations, for example, *TP53* and *RB1* in small cell

NECs and a variable genetic profile in large cell NECs[12].

Dependent on secretory products and hypersecretion of specified hormones, the pNET are called by different names. Insulinoma is the most common functional pNET (30%-40%), with an annual incidence of 1-32 cases per million population[13-17]. Functional expression means symptomatic management can be achieved effectively with surgical excision. In the case of insulinoma, tumor origin within β-cells of Islets of Langerhans means parenchymal sparing pancreatectomy can relieve symptoms of the classical Whipple's triad of hypoglycaemia (plasma glucose < 4 mmol/L), neuroglycopenic clinical signs and resolution after administration of glucose[18].

Classification of pNETs

Functionality is an important predictive factor in the presentation of pNENs[19]. Patients with nonfunctioning pNENs have larger, poorly differentiated tumors with hepatic and lymph node involvement associated with reduced survival outcomes compared to those with functioning pNENs[20]. Tumor grade is another important prognostic factor that can indicate cellular morphology according to classes of differentiation[21]. According to the World Health Organization, the pNENs are broadly classified into well-differentiated pNETs (three subtypes: G1 pNETs, G2 pNETs and G3 pNETs) and poorly differentiated pNECs (two subtypes: Small cell and large cell types and are high-grade by definition)[22]. These classifications are summarized in Table 1.

DIAGNOSTIC APPROACH

Role of serum biomarkers

Generally, serum biomarkers can be used to identify the presence of NENs with the presentation of proteins in the secretory granules, synaptic-like vesicles, or cytoplasm of neuroendocrine cells. These have low specificity as these biomarkers can also be elevated in inflammatory conditions like inflammatory bowel disease, pancreatitis, gastritis, proton pump inhibitor and steroid treatment, liver failure, and other neoplasms[12]. Specific pancreatic neuroendocrine markers[23] can be used to distinguish functional *vs* non-functional pNETs, as summarised in Table 2.

The diagnosis of NEN is established by immunohistochemical staining showing positivity for markers of neuroendocrine differentiation, including INSM1 (highly sensitive and specific), synaptophysin (highly sensitive but not specific), and chromogranin A (less sensitive than synaptophysin but highly specific)[21]. This should be followed by a stain for transcription factors to know the site of origin: For example, pNENs may express PDX1 (a transcription factor typically expressed by the β cells), ARX (transcription factor typically expressed by the α cells), and ISL1 and stain for the peptide hormones[24].

The typical (indolent) insulinomas are epigenetically similar to β cells: PDX1 positive and ARX negative[25]. They are small (below 2 cm), have a favourable prognosis after resection, and are characterized by *YY1* mutations in nearly 30% of cases. On the other hand, the rare aggressive insulinomas are ARX positive, larger (3.5-9.0 cm), have higher metastatic rates, and are characterized by genetic alternations seen characteristically in non-functioning pNENs, including *ATRX/DAXX* mutations, alternative lengthening of telomeres or *CDKN2A* deletions[25]. Hence, assessment of *ATRX/DAXX* mutations and alternative lengthening of telomeres are recommended in pNENs.

The origin, differentiation and tumorigenic mechanisms of the aggressive insulinomas are more closely related to non-functioning pNENs[25]. It is possible that they existed as non-functioning pNENs for a while before exhibiting the functional behaviour (recurrent hypoglycaemia). These tumours might have been producing insulin, though at an asymptomatic level. It is hypothesized that the tumorigenesis of aggressive AXR-positive insulinomas might have happened from a non-functioning β-cell tumor acquiring α-cell characteristics after *ATRX/DAXX* mutations and the presence of alternative lengthening of telomeres, or from a non-functioning α cell/intermediate tumor acquiring β cell characteristic of insulin secretion[25].

Role of imaging

Imaging plays an important role in the diagnosis, follow-up and assessment of response to treatment of pNETs. High-resolution computed tomography (CT) of the thorax, abdomen, and pelvis is the first line in providing radiological imaging of the location and size of the primary tumor, nodal disease, and sites of metastasis. Intravenous contrast, particularly in the late arterial phase, can prove useful for detecting NENs in the pancreas and liver[26]. Magnetic resonance imaging (MRI) has increased specificity and sensitivity compared to CT (100% specificity for pancreas and 98% for liver), and diffusion-weighted (T1/T2) imaging can aid in small hepatic metastasis identification, along with greater detail into tumor margins[19]. Positron emission tomography (PET) with F-fluorodeoxyglucose can show uptake in combination with CT/MRI.

Due to the expression of biomarkers, nuclear imaging is useful in providing functional response information of pNETs. Somatostatin Receptor (SSR) imaging is most commonly used in combination with single-photon emission computed tomography (SPECT)/CT or PET/CT and includes the use of octreotide, ^{111}In-pentereotide or Gallium-DOTA tracer[13]. Receptor positivity has important applications for the use of somatostatin analogues or targeted therapy and can measure treatment response/remission.

Staging

Following physical examination, biopsy and imaging for diagnosis, pNETs are staged, as per TNM and AJCC classifications[27], as recorded in Table 3.

Table 1 Classification of pancreatic neuroendocrine tumors, modified from World Health Organisation Classification of Neuroendocrine Neoplasms[20]

Terminology	WHO grade	Differentiation	Mitotic rate	Ki-67 index
NET	G1	Well-differentiated NET	< 2/2 mm²	< 3%
	G2		2-20/2 mm²	3%-20%
	G3		> 20%/2 mm²	> 20%
Small cell NEC	High grade	Poorly differentiated NEC	> 20/2 mm²	> 70%
Large cell NEC	High grade			

NET: Neuroendocrine tumors; NEC: Neuroendocrine carcinoma.

Table 2 Biomarkers for the diagnosis of pNEN[23]

Circulating biomarkers		
Non-specific biomarkers	Specific to functional pNEN	Specific to nonfunctional pNEN
Chromogranin A	Insulin	Pancreatic polypeptide
Pancreastatin	Glucagon	Human chorionic gonadotropin
Chromogranin B	Gastrin	Neurotensin
Neuron-specific enolase	Somatostatin	Ghrelin
Alpha fetoprotein	Vasoactive intestinal peptide	Calcitonin
	Growth hormone or GHRH	
	Adrenocorticotropic hormone	
Immunohistochemical biomarkers		
Differentiation markers	Site of origin markers	Prognostic markers
Chromogranin A	NESP55	ATRX/DAXX
Synaptophysin	PGR	SSTR2a
INSM1	PDX1	PD-L1
	ARX	
	ISL1	
Molecular biomarkers		
Circulating tumor cells (CTCs)		
Circulating cell-free DNA (cfDNA)		
Circulating microRNAs (miRNAs)		
Circulating transcripts (NETest score)		
G protein-coupled receptor-associated sorting protein 1 (GPRASP1)		
Delta-like protein 3 (DLL3)		
Tumor-associated macrophages (TAMs)		
Glucose transporter 1 (GLUT1)		

INSM1: Insulinoma-associated protein 1; NESP55: Neuroendocrine secretory protein 55; PGR: Progesterone receptor; PDX1: Pancreatic and duodenal homeobox 1; ISL-2: Insulin gene enhancer binding protein 2; ATRX/DAXX: Alpha-thalassemia/mental retardation X-linked and death domain-associated protein; SSTR2a: Somatostatin receptors 2a; PD-L1: Programmed cell death ligand 1.

Table 3 Tumor, node, metastasis and American Joint Committee on Cancer classifications for staging of pancreatic neuroendocrine tumors[27]

AJCC	TNM	Description
I	T1 N0 M0	Tumor < 2 cm, local to pancreas. No nodal disease/metastasis
II	T2 N0 M0	2 cm < Tumor < 4 cm, local to pancreas. No nodal disease/metastasis
	T3 N0 M0	Tumor > 4 cm, extended to duodenum/common bile duct. No nodal disease or metastasis
III	T1-3/4 N1 M0	Tumor extended to adjacent organs (stomach, spleen, colon/adrenals) or spread haematogenously (T4). It may present with lymph node spread (N1) but no distant metastasis
IV	Tx Nx M1	Any size (Tx), any nodal spread (Nx), but distant metastasis (M1)

Prognostic factors

SEER stages of localized, regional and distant disease can be used to categorize pNETs based on 5-year relative survival rates. Localized tumors to the pancreas report the best prognoses of up to 95%, followed by regional (adjacent organ/Lymph nodal spread; 72%) and distant metastasis to lungs, liver and bone at 23%. Overall, a 53% 5-year survival rate is reported across all stages, meaning that effective diagnosis and treatment can achieve stable disease[28].

Therapeutic approach

Treatment of pNETs falls under two main aims: Curative intent and reduced disease burden with symptomatic management. Underpinning all treatment modalities is multidisciplinary care, involving surgeons, radiologists, endocrinologists and clinical oncologists[16]. As patients with pNETs, including insulinoma, may present with varied symptoms, this is crucial to developing a personalized management plan for the patient in follow-up, maintenance and evaluating disease stability/remission status.

Surgery remains the only treatment modality with curative intent. Indications include small pNETs (< 2 cm), symptomatic non-functioning pNETs, and all functional pNETs, excluding those with positive *MEN1* genes. Techniques include parenchymal-sparing pancreatic resection, either enucleation if the tumor is superficial to the main pancreatic duct or central pancreatectomy with lymph node plucking. Larger tumors with extensive margins can be excised *via* pancreaticoduodenectomy or distal/total pancreatectomy with regional lymphadenectomy[18].

Where surgery (open/Laparoscopic/robotic) is not indicated as in poor general health, high American Society of Anesthesiologists/Eastern Cooperative Oncology Group performance score, extrahepatic metastases or pNEC, local measures can be trialled such as ablation using radiofrequency, microwave, laser or percutaneous or endoscopic cryotherapy[19]. Indications include hepatic metastases < 5 cm and ablation margins > 1 cm. Transarterial embolization can afford debulking *via* chemo/selective internal radiotherapy with Yttrium-90 isotope. Pancreatitis is the most common reported risk post-local measures. The consensus is that surgery is performed with curative intent for G1/2 tumors even with nodal/hepatic metastasis, coupled with hepatectomy or staged liver resection, followed by liver transplantation if necessary[19]. The role of whether curative intent can be achieved *via* surgery for G3 tumors over debulking is still debated in the literature.

Medical measures are indicated for symptomatic, functional pNETs or unresectable or incompletely resected tumors to suppress post-operative functionality. Recurrence can be managed symptomatically. In the case of hypoglycaemia secondary to insulinoma, this includes dietary adjustments of frequent small meals, followed by diazoxide (sulphonylurea receptor 1 blocker), which raises glucose secretion by hyperpolarising K^+-ATP (potassium-adenosine triphosphate) channels on pancreatic islet β-cells. For tumours that express somatostatin receptors, somatostatin analogs (SSAs) such as octreotide, lanreotide, or pasireotide have anti-proliferative effects. Long-acting SSA is used as standard therapy, and short-acting can be used as rescue drugs; the frequency of both can be increased if the biochemical/clinical picture does not show improvement. Pasireotide has activity against all somatostatin receptors, except SSTR4, and octreotide/Lanreotide has selective action towards SSTR2, 3 and 5, with maximal selectivity towards SSTR2[29]. According to the CLARINET study, greater progression-free survival of 32.1% is achieved by lanreotide Autogel compared to octreotide long-acting[30].

For locally advanced, unresectable or metastatic tumors that do not respond to SSA, molecular-targeted therapies can be trialled. Particularly for insulinomas, everolimus acts as an mTOR (mammalian target of rapamycin) pathway inhibitor, and sunitinib as a VEGF/PDGF (vascular endothelium growth factor/platelet-derived growth factor) inhibitor under the broader category of tyrosine kinase pathway inhibitors[13,19]. These can be used individually or in combination. Before these molecular-targeted therapies are established, neoadjuvant or adjuvant chemotherapy with alkylating agents (streptozotocin), in combination with 5-fluorouracil or doxorubicin (*e.g.*, FOLFOX/FOLFIRI) showed up to 69% response rate in tumors with high disease burden, warranting systemic mechanism of action[13,19].

Recent research is showing the promise of peptide receptor radionuclide therapy (PRRT) for the management of functioning metastatic insulinomas with refractory hypoglycaemia. This treatment modality couples the use of SSA therapy, labelled with a radioactive isotope (Luteium-177-DOTA-Tyr3-octreotate) or Indium-111 octreotide, to act as targeted systemic radiation. The choice of this 177 Lu-DOTATE isotope is preferred due to the emission of concomitant beta and gamma-rays, reduced renal and haematological toxicity and modification of C-terminal threoninol on octreotide to threonine to increase affinity to SSRT2[31]. Positive results include improved quality of life secondary to better glycaemic control (70.6%) and stable disease (23.5%)[32]. This is further supported by the fact that 81% of patients having

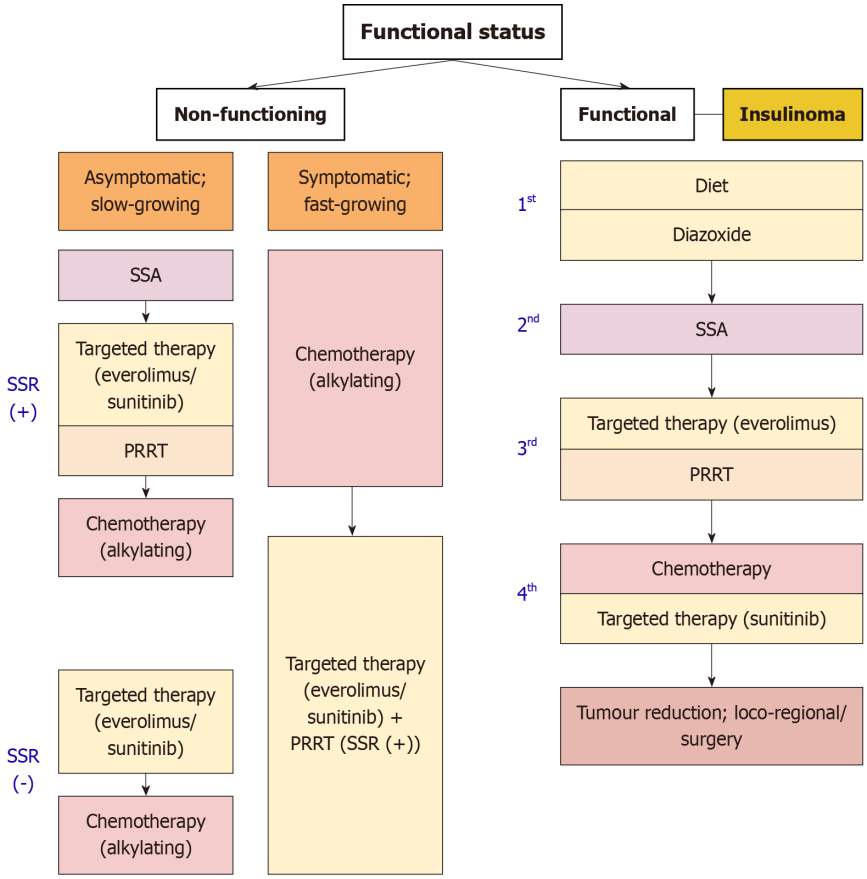

Figure 1 Management of pancreatic neuroendocrine tumors, according to functional status, adapted from European neuroendocrine tumor society 2023[33,34]. SSR: Somatostatin receptor; SSA: Somatostatin analog; PRRT: Peptide receptor radionuclide therapy.

a reduced hypoglycaemic score, 58% requiring reduced antihypoglycaemic medication and median overall survival and progression-free survival of 19.7 months (95%CI: 6.5-32.9 months) and 11.7 months (95%CI: 4.9-18.5 months), respectively, post-PRRT[29].

As per current accepted European neuroendocrine tumor society (ENETS) guidelines[33], an algorithm for the management of pNETs is summarized in Figure 1, according to functional status.

Treatment strategy

Based on the latest available guidance, the treatment strategy for non-functioning pNETs can be summarized for ease of understanding[34]. Patients with locally advanced NF-Pan-NET (stage T3 and T4) can be resected safely with low mortality and acceptable morbidity risk in expert centres. Radical local resection (R0) including portal-venous resection could be considered in selected cases. To help estimate risk of recurrence post-local measures, and guide follow-up schedules, nomograms after resection are recommended. In locally advanced or oligometastatic cases, preoperative treatment with PRRT can be beneficial in reducing tumor bulk.

For slow-growing, advanced G1-G2 non-functioning pNETs that are SSR positive, SSA is the recommended upfront treatment. In progressive G1-G2 non-functioning pNETs with SSR positivity, targeted therapy with everolimus and sunitinib are recommended. These agents should be considered in G3 progressive disease as well. PRRT may be considered second-line in non-functioning pNETs that are SSR positive. Finally, for metastatic disease, systemic chemotherapy (temozolomide in combination with capecitabine or streptozotocin + 5-FU) may be considered for patients with progressive/metastatic or symptomatic non-functioning G1-G2. For G3 metastatic non-functioning pNETs, temozolomide in combination with capecitabine can also be considered for upfront treatment[34].

CASE SUMMARY AND MANAGEMENT

A 52-year-old male presented with new recurrent, severe hypoglycaemic episodes, including nocturnal symptoms and loss of consciousness. Relevant background is a 5-year history of weight gain (body mass index 33 kg/m^2), fatigue, general malaise, loss of appetite and intermittent undiagnosed abdominal pain. Investigations revealed new-onset diabetes and a suspicious lesion on the liver and pancreas on ultrasound. The patient was prescribed insulin (Abasaglar and Humalog Kwikpen) and referred for a CT scan of the thorax, abdomen, and pelvis (TAP), which identified a large (10 cm × 9.5 cm) primary mass replacing the pancreatic tail (Figure 2A), extending diffusely into the pancreatic body. A

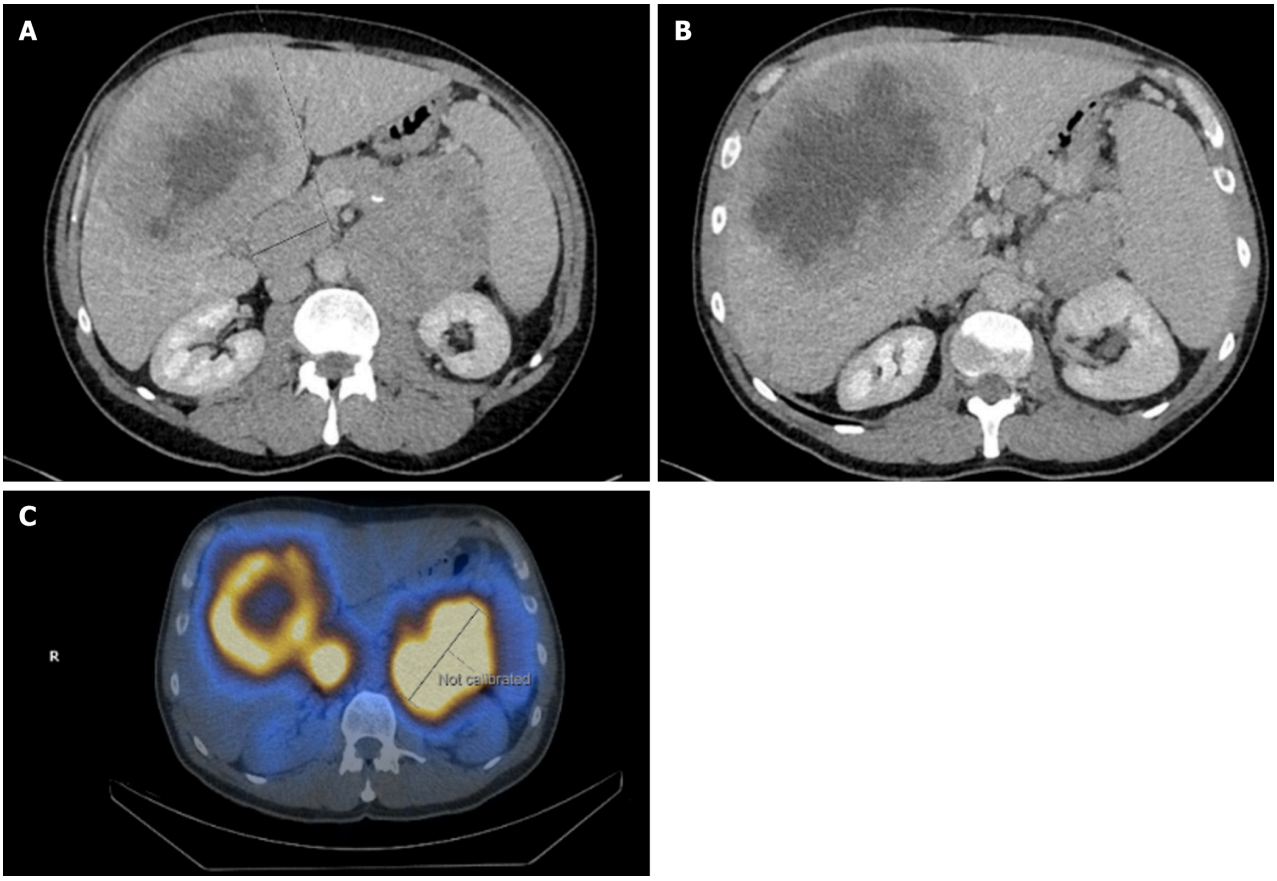

Figure 2 Initial CT-TAP confirmed 10 cm × 9.5 cm 1° mass in the pancreatic tail, extending diffusely into the pancreatic body. A and B: Evidence of metastasis – 15 cm necrotic mass in segment 8 of the liver (B); C: Increased Octreotide uptake is shown on the OctreoScan, confirming the presence of somatostatin receptor positivity.

fasting gut hormone profile suggested that the tumor was nonfunctional at the initial diagnosis. Bulky pathological lymph nodal disease was noted in the periportal territory (4.8 cm max diameter) around the superior mesenteric artery and coeliac axis. A large central necrotic mass was noted in segment 8 of the liver, measuring 15 cm (Figure 2B), with small benign cysts seen in the left hepatic lobe.

An ultrasound-guided liver biopsy of the right lobe of the liver, with histological analysis, confirmed Grade 2 NET, Ki67, 5%-10%. Planar imaging at 0004h, 0024h, and 0040h with SPECT CT Abdomen and Pelvis at 24 hours confirmed the pancreatic tail mass, periphery of large hepatic metastasis in segment 8, large portocaval node and additional 2 areas of nodal disease in the upper abdominal mesentery, with increased Octreotide uptake confirming SSTR positivity (Figure 2C). As per the guidelines, no detectable peptides on biopsy specimens and clinical picture at the time of diagnosis did not exhibit features of hormone hypersecretion syndrome, the diagnosis was non-functioning NET with metastasis[20].

In line with literature, surgical resection was not performed due to widespread liver metastasis and the advanced disease stage[35,36]. Systemic therapy was initiated to prolong the survival and for symptomatic control. As per RADIANT-2 trial[37], SSA therapy (Octreotide LAR 30 mg, subcutaneously monthly) and targeted therapy (Everolimus 10 mg, oral, for 28-day cycles). The patient developed thrombocytopenia and a generalized itchy rash with maculo-papular lesions, so Everolimus was subsequently changed to Sunitinib. 47 cycles of SSA and Everolimus were completed, and he was monitored bimonthly *via* CT-TAP (Figure 3, showing the smallest size of the primary tumor at 20 months post-treatment).

Disease progression was observed at 48 and 49 months (Figure 4), in line with a worsening clinical picture of new hypoglycaemic episodes (> 4 times weekly) and the development of biliary sepsis requiring hospitalization. Insulin was stopped due to frequent hypoglycaemic attacks during the most recent admission, but the hypoglycaemic episodes continued. He was treated with diazoxide up to 200 mg daily. Even with this and continuous intravenous glucose infusions, he developed recurrent hypoglycaemic episodes while in the hospital.

Further investigations were performed due to the evolving clinical picture from the initial presentation. A plasma C-peptide level of 6975 pmol/L with an insulin level 147 mU/L when plasma glucose was 2.4 mmol/L with a negative urine sulphonylurea screening test confirmed endogenous hyperinsulinism from a functional insulinoma. The patient was referred for peptide receptor radionuclide therapy (PRRT), in conjunction with SSA, due to disease progression despite systemic therapy, and died due to intractable hypoglycaemia and cardiac arrest.

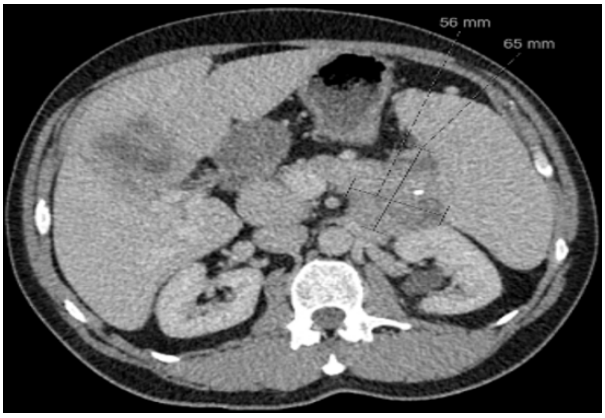

Figure 3 The smallest 1° tumor size 20 months post-initial treatment with Everolimus and somatostatin analogue therapy.

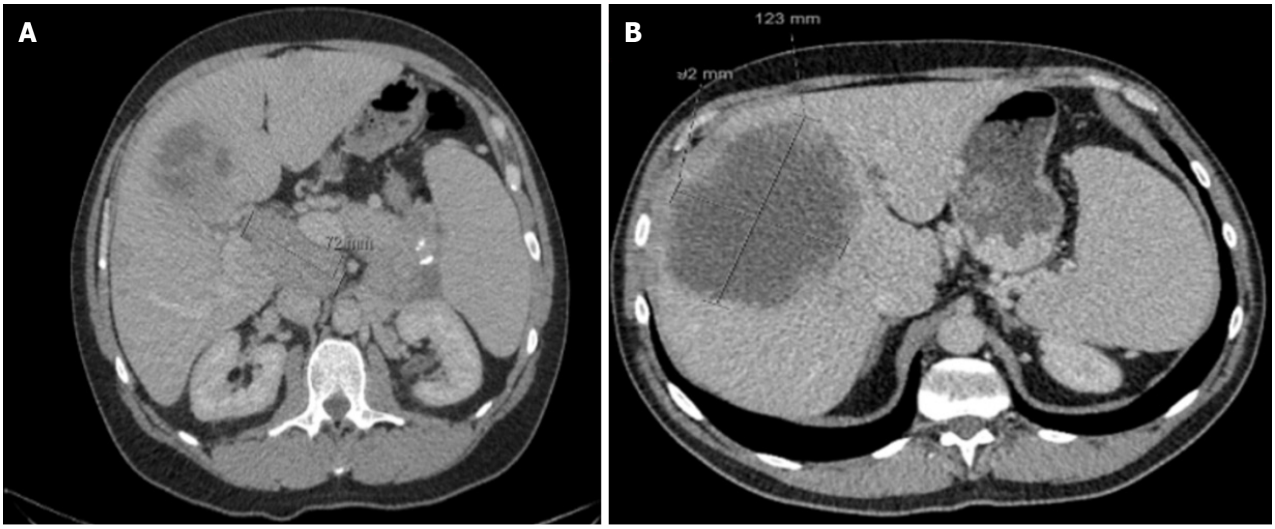

Figure 4 48 months post-treatment showing disease progression (increase in 1° tumor size and secondary hepatic metastasis and nodal disease). A and B: Increased size in secondary hepatic mass (12.3 cm × 9.2 cm) at 49 months.

DISCUSSION

The reported case highlights the initial presentation of non-functioning pNET subsequently turning out to become an insulinoma. The rate of transformation in case series is reported between 3.34% to 6.8%[38-40]. A review of the literature only highlights recent case reports with similar clinical pictures for comparison, highlighting the rarity and dilemma in clear management.

This case can be compared to that of a G3 Ki-67 index of 40% pNET with liver, lung and spinal metastasis, which was seen to turn into functional insulinoma 3 months post-diagnosis, post ineffective treatment with SSA (octreotide) to manage recurrent hypoglycaemia[40]. Glycaemic control was achieved to somewhat stable limits by Day 10 on diazoxide, octreotide and prednisolone. The case reflected reluctance from patients to try molecular-targeted therapies (everolimus/sunitinib) as a second line but also highlighted aggressive disease course in comparison to a median of 15 months in other reports[38]. The patient was given a one-off course of FOLFOX chemotherapy to manage the systemic oncological burden but had denied palliative chemotherapy and progressively declined.

A more recent case report documents a patient diagnosed with well-differentiated non-functional NET who underwent total pancreatectomy and started on an insulin regimen following surgery[41]. Two years post-surgery, the patient was identified to have recurrent disease in the mesentery and liver and received sunitinib along with PRRT. Symptoms of intermittent hypoglycaemia began a year post-treatment from recurrent disease, resulting in stopping the insulin regimen. Severe neuroglycopenic episodes and acute refractory hypoglycaemia in months following confirmed an insulinoma. Octreotide was trialled but not tolerated due to GI upset, thus, the patient's hypoglycaemia was managed with diazoxide, dexamethasone and capecitabine (for palliation). No further chemotherapy, hormonal or immunologic therapy, was suitable and refractory hypoglycaemia was managed symptomatically as and when.

Overall, these cases, including our own, report the importance of recognizing new hypoglycaemia as a red-flag symptom for an investigation into malignant insulinoma developing from a previous nonfunctional pNET. Biochemical analysis is necessary and prompts a multidisciplinary care approach to tailor individualized treatment plans as the

transformation from non-functioning to functioning pNET confers a poor prognosis and high symptomatic burden for patients. Ultimately, the prognosis is multifactorial and dependent on grade, stage and prognostic factors, but a coherent investigation can afford the most targeted treatment for tumor characteristics.

CONCLUSION

In conclusion, functioning neuroendocrine tumors are rare, and prompt management is difficult due to presenting signs and symptoms. Although extremely rare, a transformation of a nonfunctioning pNET to a functioning pNET (insulinoma) should be considered when unexplainable hypoglycaemia ensues in such a patient. Thus, this review and novel case highlight that further studies are warranted to understand the frequency of functional transformation of non-functioning pNETs. Moreover, managing refractory hypoglycaemia from malignant insulinomas is challenging, and there is an avenue for constant evidence-based recommendations of therapeutic efficacy.

FOOTNOTES

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REFERENCES

1 **Alwan H**, La Rosa S, Andreas Kopp P, Germann S, Maspoli-Conconi M, Sempoux C, Bulliard JL. Incidence trends of lung and gastroenteropancreatic neuroendocrine neoplasms in Switzerland. *Cancer Med* 2020; **9**: 9454-9461 [*RCA*] [PMID: 33078908 DOI: 10.1002/cam4.3524] [FullText] [Full Text(PDF)]
2 **Tan B**, Zhang B, Chen H. Gastroenteropancreatic neuroendocrine neoplasms: epidemiology, genetics, and treatment. *Front Endocrinol (Lausanne)* 2024; **15**: 1424839 [*RCA*] [PMID: 39411312 DOI: 10.3389/fendo.2024.1424839] [FullText]
3 **Danek E**, Kavnoudias H, McLean C, Gerstenmaier JF, Di Muzio B. Radiological Variability in Pancreatic Neuroendocrine Neoplasms: A 10-Year Single-Center Study on Atypical Presentations and Diagnostic Challenges. *Biomedicines* 2025; **13** [*RCA*] [PMID: 40002909 DOI: 10.3390/biomedicines13020496] [FullText]
4 **Magi L**, Marasco M, Rinzivillo M, Faggiano A, Panzuto F. Management of Functional Pancreatic Neuroendocrine Neoplasms. *Curr Treat Options Oncol* 2023; **24**: 725-741 [*RCA*] [PMID: 37103745 DOI: 10.1007/s11864-023-01085-0] [FullText] [Full Text(PDF)]
5 **Yan J**, Yu S, Jia C, Li M, Chen J. Molecular subtyping in pancreatic neuroendocrine neoplasms: New insights into clinical, pathological unmet needs and challenges. *Biochim Biophys Acta Rev Cancer* 2020; **1874**: 188367 [*RCA*] [PMID: 32339609 DOI: 10.1016/j.bbcan.2020.188367] [FullText]
6 **Sonbol MB**, Mazza GL, Mi L, Oliver T, Starr J, Gudmundsdottir H, Cleary SP, Hobday T, Halfdanarson TR. Survival and Incidence Patterns of Pancreatic Neuroendocrine Tumors Over the Last 2 Decades: A SEER Database Analysis. *Oncologist* 2022; **27**: 573-578 [*RCA*] [PMID: 35348774 DOI: 10.1093/oncolo/oyac049] [FullText] [Full Text(PDF)]
7 **Das S**, Dasari A. Epidemiology, Incidence, and Prevalence of Neuroendocrine Neoplasms: Are There Global Differences? *Curr Oncol Rep* 2021; **23**: 43 [*RCA*] [PMID: 33719003 DOI: 10.1007/s11912-021-01029-7] [FullText]
8 **Uhlig J**, Nie J, Gibson J, Cecchini M, Stein S, Lacy J, Kunz P, Kim HS. Epidemiology, treatment and outcomes of gastroenteropancreatic neuroendocrine neoplasms. *Sci Rep* 2024; **14**: 30536 [*RCA*] [PMID: 39690170 DOI: 10.1038/s41598-024-81518-4] [FullText]
9 **Dasari A**, Mehta K, Byers LA, Sorbye H, Yao JC. Comparative study of lung and extrapulmonary poorly differentiated neuroendocrine carcinomas: A SEER database analysis of 162,983 cases. *Cancer* 2018; **124**: 807-815 [*RCA*] [PMID: 29211313 DOI: 10.1002/cncr.31124] [FullText]
10 **Lee CS**, Perreault N, Brestelli JE, Kaestner KH. Neurogenin 3 is essential for the proper specification of gastric enteroendocrine cells and the maintenance of gastric epithelial cell identity. *Genes Dev* 2002; **16**: 1488-1497 [*RCA*] [PMID: 12080087 DOI: 10.1101/gad.985002] [FullText]

11 **Jiao Y**, Shi C, Edil BH, de Wilde RF, Klimstra DS, Maitra A, Schulick RD, Tang LH, Wolfgang CL, Choti MA, Velculescu VE, Diaz LA Jr, Vogelstein B, Kinzler KW, Hruban RH, Papadopoulos N. DAXX/ATRX, MEN1, and mTOR pathway genes are frequently altered in pancreatic neuroendocrine tumors. *Science* 2011; **331**: 1199-1203 [*RCA*] [PMID: 21252315 DOI: 10.1126/science.1200609] [FullText] [Full Text(PDF)]

12 **Uccella S**, La Rosa S, Metovic J, Marchiori D, Scoazec JY, Volante M, Mete O, Papotti M. Genomics of High-Grade Neuroendocrine Neoplasms: Well-Differentiated Neuroendocrine Tumor with High-Grade Features (G3 NET) and Neuroendocrine Carcinomas (NEC) of Various Anatomic Sites. *Endocr Pathol* 2021; **32**: 192-210 [*RCA*] [PMID: 33433884 DOI: 10.1007/s12022-020-09660-z] [FullText]

13 **Fernandez CJ**, Agarwal M, Pottakkat B, Haroon NN, George AS, Pappachan JM. Gastroenteropancreatic neuroendocrine neoplasms: A clinical snapshot. *World J Gastrointest Surg* 2021; **13**: 231-255 [*RCA*] [PMID: 33796213 DOI: 10.4240/wjgs.v13.i3.231] [FullText] [Full Text (PDF)]

14 **Dromain C**, Déandréis D, Scoazec JY, Goere D, Ducreux M, Baudin E, Tselikas L. Imaging of neuroendocrine tumors of the pancreas. *Diagn Interv Imaging* 2016; **97**: 1241-1257 [*RCA*] [PMID: 27876341 DOI: 10.1016/j.diii.2016.07.012] [FullText]

15 **Falconi M**, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, Kos-Kudla B, Kwekkeboom D, Rindi G, Klöppel G, Reed N, Kianmanesh R, Jensen RT; Vienna Consensus Conference participants. ENETS Consensus Guidelines Update for the Management of Patients with Functional Pancreatic Neuroendocrine Tumors and Non-Functional Pancreatic Neuroendocrine Tumors. *Neuroendocrinology* 2016; **103**: 153-171 [*RCA*] [PMID: 26742109 DOI: 10.1159/000443171] [FullText]

16 **Granata V**, Fusco R, Setola SV, Castelguidone ELD, Camera L, Tafuto S, Avallone A, Belli A, Incollingo P, Palaia R, Izzo F, Petrillo A. The multidisciplinary team for gastroenteropancreatic neuroendocrine tumours: the radiologist's challenge. *Radiol Oncol* 2019; **53**: 373-387 [*RCA*] [PMID: 31652122 DOI: 10.2478/raon-2019-0040] [FullText] [Full Text(PDF)]

17 **Jensen RT**, Berna MJ, Bingham DB, Norton JA. Inherited pancreatic endocrine tumor syndromes: advances in molecular pathogenesis, diagnosis, management, and controversies. *Cancer* 2008; **113**: 1807-1843 [*RCA*] [PMID: 18798544 DOI: 10.1002/cncr.23648] [FullText] [Full Text(PDF)]

18 **Park MJ**, Lee YH, Cho JH, Choi JH. Limited Palatal Muscle Resection for the Treatment of Obstructive Sleep Apnea: A Systematic Review and Meta-Analysis. *Medicina (Kaunas)* 2023; **59** [*RCA*] [PMID: 37629722 DOI: 10.3390/medicina59081432] [FullText]

19 **Ma ZY**, Gong YF, Zhuang HK, Zhou ZX, Huang SZ, Zou YP, Huang BW, Sun ZH, Zhang CZ, Tang YQ, Hou BH. Pancreatic neuroendocrine tumors: A review of serum biomarkers, staging, and management. *World J Gastroenterol* 2020; **26**: 2305-2322 [*RCA*] [PMID: 32476795 DOI: 10.3748/wjg.v26.i19.2305] [FullText] [Full Text(PDF)]

20 **Yang Z**, Shi G. Comparative outcomes of pancreatic neuroendocrine neoplasms: A population-based analysis of the SEER database. *Eur J Surg Oncol* 2022; **48**: 2181-2187 [*RCA*] [PMID: 35623986 DOI: 10.1016/j.ejso.2022.05.016] [FullText]

21 **Rindi G**, Mete O, Uccella S, Basturk O, La Rosa S, Brosens LAA, Ezzat S, de Herder WW, Klimstra DS, Papotti M, Asa SL. Overview of the 2022 WHO Classification of Neuroendocrine Neoplasms. *Endocr Pathol* 2022; **33**: 115-154 [*RCA*] [PMID: 35294740 DOI: 10.1007/s12022-022-09708-2] [FullText]

22 **Yang M**, Zeng L, Ke NW, Tan CL, Tian BL, Liu XB, Xiang B, Zhang Y. World Health Organization grading classification for pancreatic neuroendocrine neoplasms: a comprehensive analysis from a large Chinese institution. *BMC Cancer* 2020; **20**: 906 [*RCA*] [PMID: 32962649 DOI: 10.1186/s12885-020-07356-5] [FullText] [Full Text(PDF)]

23 **Bocchini M**, Nicolini F, Severi S, Bongiovanni A, Ibrahim T, Simonetti G, Grassi I, Mazza M. Biomarkers for Pancreatic Neuroendocrine Neoplasms (PanNENs) Management-An Updated Review. *Front Oncol* 2020; **10**: 831 [*RCA*] [PMID: 32537434 DOI: 10.3389/fonc.2020.00831] [FullText] [Full Text(PDF)]

24 **Moser E**, Ura A, Vogel L, Steiger K, Mogler C, Evert M, Märkl B, Scheidhauer K, Martignoni M, Friess H, von Werder A, Marinoni I, Perren A, Klöppel G, Kasajima A. ARX, PDX1, ISL1, and CDX2 Expression Distinguishes 5 Subgroups of Pancreatic Neuroendocrine Tumors With Correlations to Histology, Hormone Expression, and Outcome. *Mod Pathol* 2024; **37**: 100595 [*RCA*] [PMID: 39147030 DOI: 10.1016/j.modpat.2024.100595] [FullText]

25 **Hackeng WM**, Schelhaas W, Morsink FHM, Heidsma CM, van Eeden S, Valk GD, Vriens MR, Heaphy CM, Nieveen van Dijkum EJM, Offerhaus GJA, Dreijerink KMA, Brosens LAA. Alternative Lengthening of Telomeres and Differential Expression of Endocrine Transcription Factors Distinguish Metastatic and Non-metastatic Insulinomas. *Endocr Pathol* 2020; **31**: 108-118 [*RCA*] [PMID: 32103422 DOI: 10.1007/s12022-020-09611-8] [FullText] [Full Text(PDF)]

26 **Sundin A**, Arnold R, Baudin E, Cwikla JB, Eriksson B, Fanti S, Fazio N, Giammarile F, Hicks RJ, Kjaer A, Krenning E, Kwekkeboom D, Lombard-Bohas C, O'Connor JM, O'Toole D, Rockall A, Wiedenmann B, Valle JW, Vullierme MP; Antibes Consensus Conference participants. ENETS Consensus Guidelines for the Standards of Care in Neuroendocrine Tumors: Radiological, Nuclear Medicine & Hybrid Imaging. *Neuroendocrinology* 2017; **105**: 212-244 [*RCA*] [PMID: 28355596 DOI: 10.1159/000471879] [FullText]

27 Pancreatic Neuroendocrine Tumor Stages [Internet]. ACS; 2018 [cited 2025 Feb 12]. Available from: https://www.cancer.org/cancer/types/pancreatic-neuroendocrine-tumor/detection-diagnosis-staging/net-staging.html#:~:text=Well%2Ddifferentiated%20tumors%20(which%20include,index%20of%20more%20than%2020%25

28 Survival rates for pancreatic neuroendocrine tumor [Internet]. ACS; 2023 [cited 2025 Feb 12]. Available from: https://www.cancer.org/cancer/types/pancreatic-neuroendocrine-tumor/detection-diagnosis-staging/survival-rates.html

29 **Friebe L**, Freitag MT, Braun M, Nicolas G, Bauman A, Bushnell D, Christ E, Wild D. Peptide Receptor Radionuclide Therapy Is Effective for Clinical Control of Symptomatic Metastatic Insulinoma: A Long-Term Retrospective Analysis. *J Nucl Med* 2024; **65**: 228-235 [*RCA*] [PMID: 38164592 DOI: 10.2967/jnumed.123.265894] [FullText]

30 **Caplin ME**, Pavel M, Ćwikła JB, Phan AT, Raderer M, Sedláčková E, Cadiot G, Wolin EM, Capdevila J, Wall L, Rindi G, Langley A, Martinez S, Blumberg J, Ruszniewski P; CLARINET Investigators. Lanreotide in metastatic enteropancreatic neuroendocrine tumors. *N Engl J Med* 2014; **371**: 224-233 [*RCA*] [PMID: 25014687 DOI: 10.1056/NEJMoa1316158] [FullText]

31 **Baum RP**, Kluge AW, Kulkarni H, Schorr-Neufing U, Niepsch K, Bitterlich N, van Echteld CJ. [(177)Lu-DOTA](0)-D-Phe(1)-Tyr(3)-Octreotide ((177)Lu-DOTATOC) For Peptide Receptor Radiotherapy in Patients with Advanced Neuroendocrine Tumours: A Phase-II Study. *Theranostics* 2016; **6**: 501-510 [*RCA*] [PMID: 26941843 DOI: 10.7150/thno.13702] [FullText] [Full Text(PDF)]

32 **Antonella M**, Laura O, Stefania D, Joniada D, Matteo S. Should peptide receptors radionuclide therapy (PRRT) be considered as a treatment of choice in functioning metastatic insulinomas? A review of literature and our center experience. *Clin Transl Imaging* 2022; **10**: 425-433 [*RCA*] [DOI: 10.1007/s40336-022-00490-z] [FullText]

33 **Hofland J**, Falconi M, Christ E, Castaño JP, Faggiano A, Lamarca A, Perren A, Petrucci S, Prasad V, Ruszniewski P, Thirlwell C, Vullierme

MP, Welin S, Bartsch DK. European Neuroendocrine Tumor Society 2023 guidance paper for functioning pancreatic neuroendocrine tumour syndromes. *J Neuroendocrinol* 2023; **35**: e13318 [*RCA*] [PMID: 37578384 DOI: 10.1111/jne.13318] [FullText]

34 **Kos-Kudła B**, Castaño JP, Denecke T, Grande E, Kjaer A, Koumarianou A, de Mestier L, Partelli S, Perren A, Stättner S, Valle JW, Fazio N. European Neuroendocrine Tumour Society (ENETS) 2023 guidance paper for nonfunctioning pancreatic neuroendocrine tumours. *J Neuroendocrinol* 2023; **35**: e13343 [*RCA*] [PMID: 37877341 DOI: 10.1111/jne.13343] [FullText]

35 **Gregoire E**, Le Treut YP. Liver transplantation for primary or secondary endocrine tumors. *Transpl Int* 2010; **23**: 704-711 [*RCA*] [PMID: 20492617 DOI: 10.1111/j.1432-2277.2010.01110.x] [FullText]

36 **Cloyd JM**, Poultsides GA. Non-functional neuroendocrine tumors of the pancreas: Advances in diagnosis and management. *World J Gastroenterol* 2015; **21**: 9512-9525 [*RCA*] [PMID: 26327759 DOI: 10.3748/wjg.v21.i32.9512] [FullText] [Full Text(PDF)]

37 **Pavel ME**, Hainsworth JD, Baudin E, Peeters M, Hörsch D, Winkler RE, Klimovsky J, Lebwohl D, Jehl V, Wolin EM, Öberg K, Van Cutsem E, Yao JC; RADIANT-2 Study Group. Everolimus plus octreotide long-acting repeatable for the treatment of advanced neuroendocrine tumours associated with carcinoid syndrome (RADIANT-2): a randomised, placebo-controlled, phase 3 study. *Lancet* 2011; **378**: 2005-2012 [*RCA*] [PMID: 22119496 DOI: 10.1016/S0140-6736(11)61742-X] [FullText]

38 **Wynick D**, Williams SJ, Bloom SR. Symptomatic secondary hormone syndromes in patients with established malignant pancreatic endocrine tumors. *N Engl J Med* 1988; **319**: 605-607 [*RCA*] [PMID: 2842676 DOI: 10.1056/NEJM198809083191003] [FullText]

39 **de Mestier L**, Hentic O, Cros J, Walter T, Roquin G, Brixi H, Lombard-Bohas C, Hammel P, Diebold MD, Couvelard A, Ruszniewski P, Cadiot G. Metachronous hormonal syndromes in patients with pancreatic neuroendocrine tumors: a case-series study. *Ann Intern Med* 2015; **162**: 682-689 [*RCA*] [PMID: 25984844 DOI: 10.7326/M14-2132] [FullText]

40 **Buddhavarapu VS**, Dhillon G, Grewal HS, Soles B, Halbur L, Surani S, Kashyap R. Transformation of pancreatic nonfunctioning neuroendocrine tumor into metastatic insulinoma: A rare case report. *Clin Case Rep* 2023; **11**: e8152 [*RCA*] [PMID: 37942181 DOI: 10.1002/ccr3.8152] [FullText]

41 **Barai R**, Barai R, Kanin M, Grock S. #1697411 A New Diagnosis of Hypoglycemia: The Progression of a Non-functional Pancreatic Neuroendocrine Tumor to a Metastatic Insulinoma. *Endocr Pract* 2024; **30**: S74-S75 [DOI: 10.1016/j.eprac.2024.03.271] [FullText]

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REVIEW

Update on molecular pathogenesis of *Helicobacter pylori*-induced gastric cancer

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Abstract

Helicobacter pylori (*H. pylori*) infection is one of the most prevalent bacterial infections affecting mankind. About half of the world's population is infected with it. It causes several upper gastrointestinal diseases, including gastric cancer (GC). It has been identified as a major risk factor for GC. GC is one of the most common cancers affecting humans and the third leading cause of cancer-related deaths worldwide. *H. pylori* infection causes an inflammatory response that progresses through a series of intermediary stages of precancerous lesions (gastritis, atrophy, intestinal metaplasia, and dysplasia) to the final development of GC. Among infected individuals, approximately 10% develop severe gastric lesions such as peptic ulcer disease, 1%-3% progress to GC, and 0.1% develop mucosa-associated lymphoid tissue followed by the development of lymphoma. The bacterium has many virulence factors, including cytotoxin-associated gene A, vacuolating cytotoxin A, and the different outer membrane proteins that cause cancer by different mechanisms. These virulence factors activate cell signaling pathways such as PI3-kinase/Akt, JAK/STAT, Ras, Raf, and ERK signaling that control cell proliferation. Uncontrolled proliferation can lead to cancer. In addition, the repair of DNA damage may also be impaired by *H. pylori* infection. Reduced DNA repair in combination with increased DNA damage can result in carcinogenic mutations. The accurate identification of pathogenetic pathways is imperative for the development of targeted diagnostic markers and personalized treatments. This scoping review aims to update the readers on the role of *H. pylori* in the development of GC. It will focus on the molecular mechanisms underpinning gastric carcinogenesis in *H. pylori* infection. It will highlight the interaction between bacterial virulence factors and host cellular pathways, providing insights into potential

therapeutic targets and preventive strategies.

Key Words: *Helicobacter pylori*; Molecular pathogenesis; Gastric cancer; DNA repair; Mutations

Core Tip: *Helicobacter pylori* (*H. pylori*) is a major risk factor for gastric cancer (GC), the third leading cause of cancer-related deaths. *H. pylori* triggers chronic inflammation, progressing through precancerous stages (gastritis, atrophy, intestinal metaplasia, dysplasia) to GC. Virulence factors like cytotoxin-associated gene A and vacuolating cytotoxin A activate oncogenic signaling pathways (PI3K/Akt, JAK/STAT, Ras/Raf/ERK), promoting uncontrolled cell proliferation and impairing DNA repair, leading to carcinogenic mutations. While 1%-3% of infected individuals develop GC, understanding these molecular mechanisms is crucial for identifying diagnostic markers and developing targeted therapies. This review explores *H. pylori*'s role in gastric carcinogenesis, emphasizing bacterial-host interactions and potential preventive strategies.

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INTRODUCTION

Gastric cancer (GC) is a major global public health challenge, and *Helicobacter pylori* (*H. Pylori*) is identified as a key etiological factor in about 89% of non-cardia GCs. *H. pylori* infection is one of the most widespread bacterial infections globally, affecting approximately half of the world's population[1-3]. This Gram-negative bacterium colonizes the gastric mucosa and is a leading cause of various upper gastrointestinal (GI) diseases, including chronic gastritis, peptic ulcers (PU), and GC[4-6]. Among these, GC remains a significant global health burden, ranking as the third leading cause of cancer-related deaths worldwide. *H. pylori* has been classified as a Group I carcinogen by the World Health Organization, underscoring its critical role in the development of GC[7]. Despite its high prevalence, only a subset of infected individuals progress to severe outcomes, such as GC or mucosa-associated lymphoid tissue lymphoma, highlighting the complex interplay between bacterial virulence factors, host immune responses, and environmental influences[4-6].

The pathogenesis of *H. pylori*-induced GC involves a multistep process characterized by chronic inflammation and progressive mucosal injury. The infection initiates a series of precancerous lesions, including gastritis, atrophy, intestinal metaplasia, and dysplasia, which can eventually lead to malignant transformation[8-10]. The principal virulence factors of *H. pylori*, such as vacuolating cytotoxin A (VacA) and cytotoxin-associated gene A (CagA), play pivotal roles in disrupting host cellular signaling pathways, including PI3K/Akt, JAK/STAT, and Ras/Raf/ERK, which regulate cell proliferation and survival[11]. Additionally, *H. pylori* infection impairs DNA repair mechanisms and increases DNA damage, creating a mutagenic environment that drives carcinogenesis[12-14]. These molecular insights have opened new avenues for understanding the mechanisms underlying gastric carcinogenesis and identifying potential therapeutic targets[15-17].

This review aims to provide an updated overview of the molecular mechanisms by which *H. pylori* contributes to the development of GC. It will explore the intricate interactions between bacterial virulence factors and host cellular pathways, shedding light on the processes that drive malignant transformation. By elucidating these pathogenetic pathways, this review seeks to highlight potential diagnostic markers and personalized treatment strategies, offering hope for improved prevention and management of *H. pylori*-associated gastric diseases, in particular, GC.

METHODOLOGY

This scoping review was conducted following the structured framework developed by Arksey and O'Malley[18], which provides a systematic and transparent approach to mapping key concepts, evidence, and gaps in a broad research area. The framework consists of five key stages: (1) Identifying the research question; (2) Identifying relevant studies; (3) Selecting studies; (4) Charting the data; and (5) Collating, summarizing, and reporting the results. Each stage was carefully executed to ensure a comprehensive and rigorous review of the literature on the role of *H. pylori* in GC development, with a particular focus on molecular pathogenesis.

The research question guiding this review was: What are the molecular mechanisms by which *H. pylori* infection contributes to the development of GC, and how can these mechanisms inform diagnostic and therapeutic strategies? To address this question, a systematic search was conducted across three major electronic databases-PubMed, Scopus, and Web of Science-to identify relevant studies published within the last decade. The search strategy employed a combination of Medical Subject Headings (MeSH) terms and keywords, including "*H. pylori*", "*Helicobacter pylori*", "gastric cancer", "molecular pathogenesis", "CagA", "VacA", "signaling pathways", and "DNA damage". Boolean operators (AND, OR)

were used to refine the search and ensure the inclusion of studies addressing both bacterial virulence factors and host cellular responses.

Following the database search, studies were screened based on predefined inclusion and exclusion criteria. Inclusion criteria encompassed peer-reviewed articles published in English, focusing on the molecular mechanisms of *H. pylori*-induced gastric carcinogenesis and providing insights into bacterial virulence factors, host signaling pathways, or therapeutic targets. Exclusion criteria included studies unrelated to *H. pylori* or GC, reviews without original data, and studies published before the 10-year period. The selected studies were then carefully scrutinized to extract key information, such as study design, molecular pathways, virulence factors, and therapeutic implications. Data were collated and synthesized to identify common themes, emerging trends, and gaps in the current understanding of *H. pylori*-associated GC. Finally, the findings were summarized and reported, providing a comprehensive overview of the molecular mechanisms underlying *H. pylori*-induced gastric carcinogenesis and their potential clinical applications.

BRIEF EPIDEMIOLOGY OF *H. PYLORI* IN ASIA

In Asia, the prevalence of *H. pylori* infection varies significantly across countries and even within regions of the same country[1-3]. For instance, Pacific Asian populations such as Chinese, Korean, and Japanese have notably high rates of both *H. pylori* infection and GC compared to other parts of the world. Conversely, countries like Singapore, Malaysia, Taiwan, and Vietnam demonstrate an intermediate risk due to a lower prevalence of both *H. pylori* and GC[19].

In South Asia and Middle East, the epidemiology of *H. pylori* infection presents a distinct scenario, characterized by a high prevalence of *H. pylori* infection but a relatively low number of GC cases. This contrast might be explained by the genetic diversity of the pathogen and variances in susceptibility among different ethnic groups[20].

H. PYLORI VIRULENCE FACTORS AND THEIR MECHANISMS

H. pylori infection is strongly associated with GI conditions like gastritis, gastric ulcer (GU), duodenal ulcer, and GC, and is classified as a class I carcinogen by the WHO. Numerous virulence factors of *H. pylori* have been identified, with CagA and the cag PAI playing pivotal roles in the pathogenesis of *H. pylori*-related diseases, including acute gastritis, GU, and GC. The key virulence factors of *H. pylori* that contribute to the pathogenicity and GC development, and their mechanisms are listed in Table 1. Other virulence factors, such as VacA and SabA, also mediate *H. pylori*'s pathogenicity. CagA, encoded by the cagA gene, is prevalent in East Asian strains, contributing to epithelial and severe histological damage compared to Western strains. VacA, encoded by the vacA gene, induces cytoplasmic vacuolation in gastric epithelial cells, with toxin activity levels affected by different genotypic combinations. Some other virulence factors involved in the pathogenicity of *H. pylori* infection and their mechanisms are shown in Table 2. The s1am1 and s1bm1 genotypes are highly virulent and associated with acute gastritis, PU, and GC. Upon entering the gastric lumen, *H. pylori* undertakes four essential activities for effective colonization and prolonged infection: (1) Surviving in gastric acidic milieu; (2) Approaching epithelial cells by flagella-mediated motility; (3) Attachment to host epithelial cells *via* bacterial adhesins interacting with epithelial cell surface receptors; and (4) Induction of cellular injury by the toxins' secretion[21-23] (Figure 1).

H. pylori subsists within the acidic gastric environment by employing a method which regulates periplasmic pH through the activity of urease. The urease gene cluster comprises seven genes, which encode the catalytic components (ureA/B), an acid-sensitive urea transporter (ureI), and helper proteins for enzyme assembly (ureE-H). In *H. pylori*, intracellular urease function is vital for surviving acidic conditions. The ureI channel controls this by allowing urea influx solely in low-pH environments, avoiding harmful pH increases when conditions are less acidic. Outside the cell, urease converts urea into carbon dioxide and ammonia, generating ammonium hydroxide. This reaction buffers the surrounding acidic environment, protecting the bacteria as they traverse the harsh gastric fluid. *H. pylori* migrates toward the basal aspect of the stomach epithelium, where the pH is near 7.0, aided by the action of 4-7 polar flagella. Increased motility, as observed in certain *H. pylori* strains, leads to increased bacterial density and a florid inflammatory response in the mucosa of the gastric wall, indicating the flagellum's role as a colonization and virulence factor in the initial phase[24-27].

Bacterial adhesins interact with host cell receptors, facilitating attachment and protecting against displacement by forces like peristalsis and gastric emptying. Notably, BabA and SabA are well-studied adhesins. Many other virulence factors, including neutrophil-activating protein (NAP) and heat shock protein 60 (Hsp60), also play important roles in mediating tissue injury. NAP stimulates the production of oxygen radicals by neutrophils, leading to tissue damage and the release of inflammatory mediators like interleukins (IL)-8, MIP-1a, and MIP-1b, linked with mononuclear cell and neutrophil infiltration into the stomach mucosa following infection by *H. pylori*. Hsp60 triggers nuclear factor kappa B (NF-κB) activation *via* TLR2 and MAP kinase signaling pathways, stimulating IL-8 release from human monocytes. Elevated anti-Hsp60 antibody levels are commonly observed in *H. pylori*-infected individuals, and their concentrations correlate with the severity of gastritis or GC. The prevalence of CagA-positive *H. pylori* is approximately 60% in Western countries and approaches 90% in Asian populations. The cagPAI, a 40 kb chromosomal DNA segment, harbors over 30 genes, including six homologous to the type IV secretion system (T4SS), facilitating the injection of CagA into the host gastric cell cytoplasm[28-31].

Once translocated, CagA interacts with phosphatidylserine, localizing to the inner surface of the plasma membrane. Upon phosphorylation, CagA interacts with the phosphatase SHP-2, modulating cellular processes such as adhesion, motility, and spreading. Beyond this, CagA triggers cytoskeletal remodeling, alters proliferation, and enhances IL-8 secretion from gastric epithelial cells. Independent of phosphorylation, CagA engages with the hepatocyte growth factor

Table 1 Key virulence factors involved in *Helicobacter pylori* infection

Key virulence factors	Mechanisms of action
CagPAI and CagA	CagPAI encodes the type IV secretion system and effector protein CagA. CagA is translocated into epithelial cells, where it phosphorylates and triggers signaling cascades associated with gastric cancer pathogenesis
VacA	VacA is a secreted toxin that induces vacuolation in host cells. It affects T cell proliferation, mitochondrial function, apoptosis, IL-8 release, and autophagy. Genetic polymorphisms in VacA influence its activity and are associated with the risk of gastric cancer
Urease	Urease hydrolyzes urea to neutralize stomach acid and maintain an optimal pH for bacterial survival
Flagella	Flagella facilitate bacterial movement and colonization. They also contribute to biofilm formation and modulate the immune response by inducing the release of IL-8
Outer membrane proteins (OMPs)	OMPs like BabA, SabA, and OipA interact with host receptors, promoting long-term colonization, chronic inflammation, and IL-8 secretion

CagA: Cytotoxin-associated gene A; BabA: Blood group antigen-binding adhesion A; SabA: Sialic acid-binding adhesion A; VacA: Vacuolating cytotoxin gene A; IL: Interleukin.

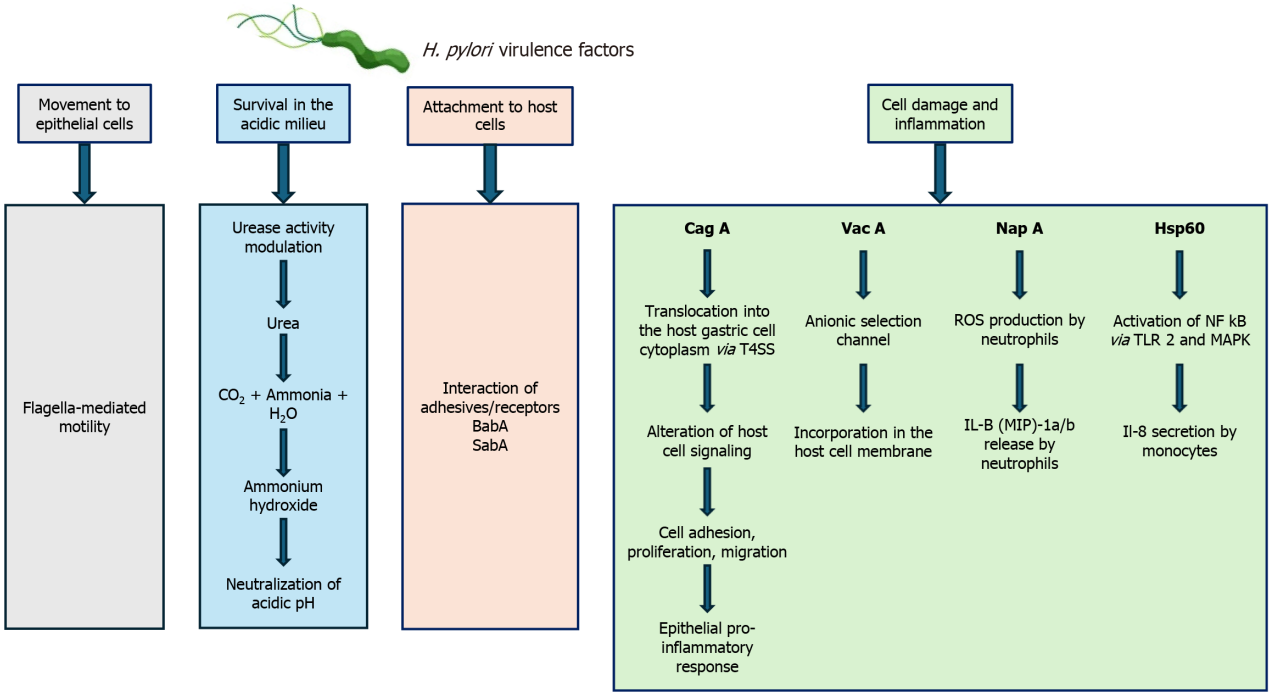

Figure 1 The key virulence factors involved in the pathogenicity of *Helicobacter pylori* infection. BabA: Blood group antigen-binding adhesion A; SabA: Sialic acid-binding adhesion A; NAP: Neutrophil activating protein; Hsp: Heat shock protein; VacA: Vacuolating cytotoxin gene A; CagA: Cytotoxin-associated gene A; T4SS: Type IV secretion system; ROS: Reactive oxygen species; IL: Interleukin; MIP: Macrophage inflammatory protein.

receptor Met, resulting in sustained stimulation of the PI3K/Akt pathway. This signaling cascade drives gastric epithelial proliferation and fosters a pro-inflammatory state linked to chronic gastritis and GC *via* the activation of NF-κB and β-catenin pathways (Figure 2; Table 3). Furthermore, *H. pylori* CagA upregulates DNMT1 expression *via* the AKT–NF-κB pathway, leading to hypermethylation and inactivation of tumor suppressor genes like MGMT in stomach epithelial cells. This mechanism contributes to GC development by promoting aberrant hypermethylation of promoter CpG islands of tumor suppressor genes[28-32].

The mammalian Hippo tumor suppressor signaling pathway plays a critical role in regulating the size and homeostasis of developing organs. Within this pathway, Yes-associated protein (YAP) serves as a key downstream effector, influencing various cellular functions such as proliferation, differentiation, and migration of gastric epithelial cells. Elevated YAP activity or overexpression has been strongly associated with enhanced cell proliferation and anti-apoptotic effects in malignancies. This upregulation shows a significant correlation with disease advancement across multiple cancer types in human patients[33]. Li *et al*[34] conducted insightful experiments revealing that *H. pylori*'s injection of CagA into cultured stomach epithelial cells stimulates the oncogenic YAP pathway. This leads to a decrease in E-cadherin expression and an upregulation of the epithelial-mesenchymal transition program, thereby fostering gastric carcinogenesis[35-38].

Table 2 Some other virulence factors involved in the pathogenicity of *Helicobacter pylori* infection

Virulence factors	Mechanisms of action
Lipopolysaccharide	Triggers several signaling pathways
	Induces several inflammatory responses
	Induces immune responses
	Disrupts the mucus secretion
	Shielding the organism against toxic materials
Phospholipase	Activates signaling pathways (*e.g.*, ERK1/2)
	Trigger chronic inflammation
	Enhances bacterial colonization and survival
	Involved in the degradation of lipids and damage to the mucus layer
Heat shock proteins	Enhance adherence to epithelial surfaces
	Involved in urease activation
	Control apoptosis and autophagy
	Help to maintain the structure and properties of the effector proteins
	Protect the cell from reactive oxygen species (ROS)
	Induce the production and release of IL-8, TNF-α and COX-2
Arginase	Prevents bacterial killing
	Prevents T-cell proliferation
	Impair immune responses
	Stimulate apoptosis
	Help the *H. pylori* to withstand the acidic environment
Superoxide dismutase	Protects the cell from ROS
	Enhances colonization
	Inhibits the production of cytokines
	Stimulates macrophage activation
γ-glutamyl-transferase	Facilitates apoptosis and necrosis
	Induces the release of pro-inflammatory proteins
	Induces the release of ROS
	Stimulates DNA damage
Cholesteryl α-glucosyltransferase (αCgT)	Shields *H. pylori* from immunological attack
	Stimulates the production of pro-inflammatory proteins (*e.g.*, IL-8)
	Enhances bacterial growth and its resistance to antibiotics

TNF-α: Tumor necrosis factor alpha; IL: Interleukin; *Helicobacter pylori*: *H. pylori*.

VacA stands as another pivotal virulence factor associated with *H. pylori*, recognized for its multifaceted impact on host cells, including vacuolization, necrosis, and apoptosis. The VacA complex possesses the ability to integrate into the host cell membrane, exhibiting characteristics of an anionic selective channel. The VacA toxin acts as a channel-forming protein that enables bicarbonate and organic anions to enter host cells, supporting *H. pylori* colonization by exporting nutrients that promote bacterial growth. Previous studies indicate that VacA can localize to multiple cellular compartments. The toxin is internalized *via* endocytosis, reaching endosomal membranes, while extracellularly applied VacA directly targets mitochondria, triggering cytochrome C release and apoptotic cell death. Furthermore, VacA activates endoplasmic reticulum stress pathways, promoting both autophagy and apoptosis. *H. pylori*-driven epithelial cell apoptosis may play a dual role in disease pathogenesis, contributing to both acute gastric damage and the long-term progression to atrophy and malignancy[39,40].

Table 3 Signaling pathways activated by *Helicobacter pylori* infection that promote uncontrolled cell proliferation

Signaling pathways	Molecular mechanisms involved in gastric cancer induced by *H. pylori*
STAT3 pathway	*H. pylori* activates the STAT3 pathway through upregulation of IL-6, CagA-mediated SHP-2 activation, and TLR2 interaction. STAT3 regulates downstream target genes involved in cellular processes such as development, proliferation, differentiation, EMT, invasion, and metastasis
NF-κB pathway	*H. pylori* activates NF-κB through direct activation by CagA, IKK kinase, and upregulation of pro-inflammatory factors. NF-κB transcriptionally regulates genes involved in cell cycle progression, apoptosis inhibition, and cross-regulates with other tumor signaling pathways
Wnt/β-catenin	*H. pylori* activates the Wnt/β-catenin pathway through CagA-mediated accumulation and nuclear translocation of β-catenin. Activation of this pathway disrupts cell cycle regulation, inhibits apoptosis, induces EMT, and promotes tumor cell proliferation, motility, and invasion. Cross-regulation between Wnt/β-catenin and other pathways enhances oncogenic effects
Miscellaneous signaling pathways	*H. pylori* activates additional signaling pathways including the MAPK pathway (ERK, JNK, p38), PI3K/Akt pathway, Hippo pathway, and various other pathways (HGF/Met, TGF-β, Hedgehog, Notch). These pathways are involved in regulating proliferation, survival, migration, invasion, differentiation, apoptosis, stem cell properties, microRNA map, and exhibit complex cross-regulatory interactions with each other and with the classical pathways

Helicobacter pylori: H. pylori.

ROLE OF OXIDATIVE STRESS AND CHRONIC INFLAMMATION

Persistent inflammation plays a pivotal role in the development of numerous malignancies, with *H. pylori*-associated GC representing a prominent example. The infection initiates inflammatory cascades through multiple mechanisms, affecting both the gastric epithelium (the primary site of bacterial contact) and recruited immune cells including neutrophils, macrophages, and lymphocytes that infiltrate the infected tissue[41-43].

Oxidative stress, characterized by an increase in reactive oxygen species (ROS) production, plays a pivotal role in causing damage to gastric epithelial cells and promoting carcinogenesis. ROS can induce oxidative DNA damage, leading to the formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG), a marker associated with DNA damage linked to cancer development[44-48].

As essential effector cells of innate immunity, neutrophils perform critical antimicrobial functions through chemotaxis, phagocytosis, and pathogen elimination. They achieve this through the production of antimicrobial substances like oxidants, proteinases, and antimicrobial peptides[49-51]. ROS and reactive nitrogen species (RNS) produced by neutrophils serve a dual function. They act as antimicrobial agents by directly killing microbial pathogens and also regulate the physiological functions of neutrophils by modulating various molecular pathways. However, in the gastric mucosa infected by *H. pylori*, ROS and RNS fail to eliminate the bacterium[52]. *H. pylori* can withstand oxidative stress through defense mechanisms involving the production of antioxidant enzymes such as NapA, catalase, and superoxide dismutase. Interestingly, *H. pylori* may even contribute to increased oxidative stress in gastric epithelial cells. *H. pylori*-derived CagA induces the expression of spermine oxidase, an enzyme involved in converting spermine to spermidine, resulting in the production of H_2O_2 as a by-product. Elevated levels of H_2O_2 can lead to ROS accumulation through mitochondrial membrane depolarization and activation of caspase-mediated apoptosis. Elevated levels of ROS/RNS induce multiple forms of DNA lesions, such as point mutations, adduct formation, and single- or double-strand breaks (DSBs). Notably, 8-OHdG, a prominent oxidative DNA lesion, is markedly upregulated in GC tissues. APE1 serves as a critical mediator in oxidative stress responses, participating in both gene expression modulation and the base excision repair (BER) pathway. *H. pylori* infection can modulate APE1 function, with increased oxidative stress upregulating APE1 levels initially to repair DNA damage, but chronic infection eventually inhibits APE1 expression, leading to genetic instability[53,54].

The adaptive immune response to *H. pylori* involves complex molecular pathways, where locally produced cytokines are essential for maintaining persistent inflammation. During infection, the gastric mucosa shows marked upregulation of Th1-associated [including interferon-γ (IFN-γ)] and Th17-associated cytokines (such as IL-17A and IL-21), whose expression is modulated by antigen-presenting cell-derived mediators, particularly IL-12 and IL-23[55-58].

Studies by D'Elios *et al*[59] demonstrated the local production of anti-*H. pylori* IgA and IgG, along with a specific response of Th1 effectors in the gastric antrum of infected patients, leading to increased synthesis of IFN-γ, tumor necrosis factor alpha (TNF-α), and IL-12. This immune response may contribute to the development of PU or *H. pylori*-related gastric B-cell lymphoma. Circulating anti-*H. pylori* IgG and IgA antibodies provide reliable serological markers for detecting bacterial infection, representing a convenient non-invasive approach for tracking *H. pylori* status in patients with premalignant gastric conditions, including atrophy, metaplasia, and dysplasia. Transforming growth factor-β1 (TGF-β1) functions as a potent suppressor of Th1-mediated immunity, modulating inflammatory responses during chronic infection. In *H. pylori*-infected gastric tissue, elevated expression of Smad7, a negative regulator of TGF-β1 signaling, inhibits the TGF-β1 regulatory cascade. This leads to heightened levels of IFN-γ and T-bet, exacerbating the Th1 immune response and tissue damage. T cell-derived cytokines, such as IL-21 and IL-17A, augment the production of matrix metalloproteinases (MMPs), leading to epithelial damage and mucosal ulceration. IL-21, found at elevated levels in *H. pylori*-infected gastric mucosa, stimulates the production of MMP-2 and MMP-9 in gastric epithelial cells, exacerbating tissue damage. IL-17A, another cytokine overproduced during *H. pylori* infection is positively correlated with GC

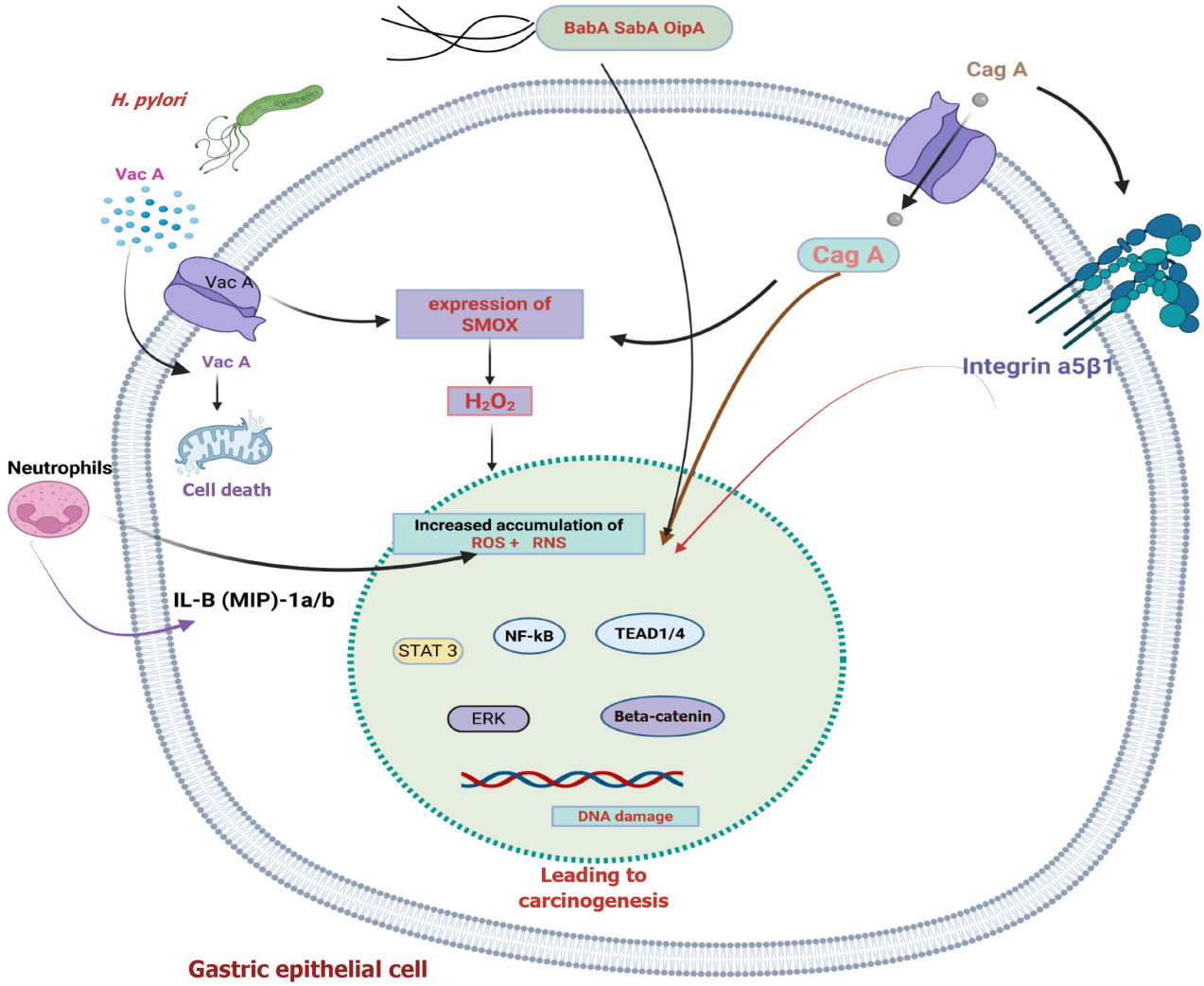

Figure 2 The main signaling pathways implicated in the pathogenesis of gastric cancer development by *Helicobacter pylori* virulence factors. This figure was created by BioRender.com (Supplementary material).

progression and invasiveness. It stimulates the production of inflammatory mediators and MMPs, propagating mucosal inflammation and destruction[59].

Although *H. pylori* triggers inflammatory responses, it demonstrates remarkable persistence in the gastric mucosa, often surviving for decades. Emerging research reveals that bacterial cholesterol-α-glucosyltransferase activity depletes cellular cholesterol stores in infected epithelial cells. This enzymatic action disrupts IFN-γ-mediated signaling, thereby suppressing host inflammatory defenses and facilitating bacterial immune evasion[60].

HOST RESPONSES AND GENETIC SUSCEPTIBILITY

Host genetic polymorphisms, particularly in genes associated with proinflammatory responses such as IL-1β, IL-1RN, IL-8, IL-10, and TNF-α, play a significant role in influencing the severity of *H. pylori*-related diseases. These genetic variations can modulate the host immune response to *H. pylori* infection, impacting disease progression and clinical outcomes[61]. IL-1β is a key proinflammatory cytokine involved in the regulation of immune responses. Polymorphisms in the IL-1β gene have been linked to altered IL-1β expression levels, affecting the intensity of the inflammatory response to *H. pylori* infection. IL-1 receptor antagonist (IL-1RN) is a natural inhibitor of IL-1β activity. Genetic variations in the IL-1RN gene can influence the balance between IL-1β and its antagonist, thereby modulating the inflammatory response and disease outcome in *H. pylori*-infected individuals. IL-8 is a chemokine that plays a crucial role in recruiting neutrophils and other inflammatory cells to the site of infection. Genetic polymorphisms in the IL-8 gene can affect its expression levels, influencing the magnitude of the inflammatory response and tissue damage in *H. pylori*-related diseases. IL-10 is an anti-inflammatory cytokine that regulates immune responses and helps prevent excessive tissue damage. Variations in the IL-10 gene can impact its expression levels, thereby modulating the balance between proinflammatory and anti-inflammatory responses during *H. pylori* infection. TNF-α is another key proinflammatory cytokine involved in the immune response to *H. pylori*. Genetic polymorphisms in the TNF-α gene can influence TNF-α production, affecting the intensity

of the inflammatory response and disease severity in *H. pylori*-infected individuals[61,62].

Overall, host genetic polymorphisms in genes related to proinflammatory responses play a crucial role in shaping the immune response to *H. pylori* infection and determining the severity of associated diseases. Understanding these genetic factors can provide valuable insights into disease pathogenesis and aid in the development of personalized treatment strategies for *H. pylori*-related conditions.

DYSREGULATION OF DNA REPAIR PATHWAYS

H. pylori impairs host DNA repair mechanisms through multiple pathways, creating genomic instability that drives gastric carcinogenesis. The bacterium's virulence factors, particularly CagA and VacA, play central roles in this process.

CagA disrupts key DNA damage response proteins such as p53 and ATM/ATR, which are critical for detecting and repairing DNA DSBs. By inducing oxidative stress through ROS and RNS, *H. pylori* causes chronic inflammation, further damaging DNA while simultaneously suppressing repair mechanisms like BER and mismatch repair (MMR). VacA exacerbates this by forming pores in mitochondrial membranes, leading to mitochondrial dysfunction and additional ROS production. Moreover, *H. pylori* infection promotes epigenetic silencing of DNA repair genes (*e.g.*, MLH1 and MGMT) *via* hypermethylation, impairing error correction. The combined effects of direct DNA damage, suppressed repair pathways, and inflammation-induced mutations create a permissive environment for oncogenic mutations in genes like TP53 and CDH1 (E-cadherin), accelerating the progression from chronic gastritis to GC. Thus, *H. pylori* acts as a biological carcinogen not only by inducing inflammation but also by systematically disrupting the host's genomic safeguards[63].

Dysregulation of DNA repair pathways, including deficiencies of enzymes like PMS2 and ERCC1, has been implicated in the development of *H. pylori*-associated GC. Understanding the role of these DNA repair enzymes in the pathogenesis of GC associated with *H. pylori* infection can provide insights into potential therapeutic targets for preventing or treating this malignancy[64,65]. Further investigation is necessary to uncover the mechanisms underlying the dysregulation of DNA repair pathways in *H. pylori*-infected gastric tissues and their contribution to cancer development. *H. pylori* infection triggers both inflammatory responses and genotoxic effects in host cells, leading to direct and indirect DNA lesions, including oxidative stress-induced damage and DSBs. Consequently, genetic and/or epigenetic disruptions alter the selection of DNA repair pathways, leading to inaccurate DNA repair, genomic instability, and chromosomal aberrations, all of which can promote gastric carcinogenesis. The cellular DNA damage response employs multiple repair mechanisms, including MMR, BER, nucleotide excision repair, homologous recombination (HR), and both canonical (NHEJ) and alternative end-joining pathways. These repair systems operate in concert with checkpoint signaling that induces cell cycle arrest or apoptosis when DNA damage cannot be properly corrected. Mounting evidence indicates that *H. pylori* infection dysregulates DNA repair processes through either transcriptional modulation of repair genes or direct functional interference with repair machinery.

The MMR pathway is a crucial mechanism for maintaining genome stability by correcting errors that occur during DNA replication. Dysfunctions in MMR are associated with various diseases, including hereditary non-polyposis colorectal cancer and brain tumors. In human cells, several MMR proteins have been identified. The hMSH2-hMSH6 complex (hMutSα) primarily recognizes base-base mismatches and small insertion-deletion loops, while MutSβ (hMSH2-hMSH3 complex or hMutSβ) targets larger insertion-deletion loops. These complexes, along with MutL homologs (hMLH1, hMLH3, hPMS1, and hPMS2), form the MMR machinery. While hMutLα (hMLH1-hPMS2 complex) is essential for MMR, the functions of hMutLβ and hMutLγ are less understood[66-69].

In eukaryotic cells, defects in DNA MMR are detected as microsatellite instability (MSI), characterized by alterations in simple sequence repeats. MSI is considered a hallmark of MMR deficiencies and is a reliable biomarker for stomach cancer. Notably, MSI-positive GCs demonstrate significantly higher *H. pylori* colonization rates than their MSI-negative counterparts. This clinical observation suggests potential bacterial-mediated dysregulation of MMR mechanisms during gastric carcinogenesis[70].

Studies have investigated the impact of *H. pylori* infection on the MMR pathway in GC cell lines. Studies revealed a significant reduction in MLH1, PMS1, PMS2, MSH2, and MSH6 protein levels following *H. pylori* infection, a phenomenon not mediated by the CagA virulence factor. While MSH2 and MSH6 mRNA expression was correspondingly suppressed, MLH1 transcript levels remained unaffected. Importantly, the expression of MLH1 and MSH2 returned to normal levels after *H. pylori* eradication, indicating reversible inhibition of MMR gene expression. Further investigations in chronically infected patients with *H. pylori* before and after eradication treatment showed that bacterial eradication increased MLH1 and MSH2 expression, suggesting that chronic *H. pylori* infection may have a negative impact on MMR in gastric epithelium, leading to mutation accumulation. Consistent with these findings, studies on human gastric tissue samples revealed lower MLH1-positive epithelial cell nuclei in *H. pylori*-positive patients compared to uninfected individuals.

Both cell culture and animal model studies consistently showed that *H. pylori* infection reduces MMR capacity in gastric epithelial cells, evidenced by diminished expression of MMR genes and their protein products, along with impaired repair function. This decrease was not dependent on bacterial virulence factors and led to increased genetic instability. Moreover, *H. pylori* infection induced the accumulation of DSBs in gastric cells, primarily repaired through error-prone NHEJ rather than HR. This shift in repair mechanism was mediated by the upregulation of NHEJ-related genes and the downregulation of HR-related genes[71,72].

A study identified the upregulation of long, noncoding RNA SNHG17 by *H. pylori* infection, which increased DSBs by promoting NHEJ over HR repair. SNHG17-mediated recruitment of NONO, involved in NHEJ, and its role as a decoy for

miR-3909, which regulates HR, contributed to chromosomal abnormalities associated with GC development. Overall, these findings demonstrate that *H. pylori* infection induces dysregulation of the MMR pathway, leading to increased genetic instability and promoting gastric carcinogenesis[72,73].

MOLECULAR HETEROGENEITY OF GC

GC is a molecularly heterogeneous disease, and *H. pylori*-induced tumors exhibit distinct molecular features that influence both pathogenesis and treatment response[74]. The TCGA study identified four major GC subtypes, with *H. pylori*-associated cancers most commonly falling into the chromosomal instability (CIN) group, characterized by TP53 mutations and intestinal-type histology[75]. Similarly, the ACRG classification by Cristescu *et al*[76] linked *H. pylori*-driven cancers to the MSS/TP53- and MSS/TP53+ subtypes, both associated with worse outcomes compared to MSI-high tumors. *H. pylori*-induced GC is most commonly associated with intestinal-type histology, CIN, and specific molecular changes such as TP53 mutations, epigenetic silencing, and inflammatory gene signatures. Understanding these molecular traits can help refine treatment strategies and identify which patients benefit most from targeted therapies or immune modulation.

POTENTIAL THERAPEUTIC TARGETS AND PREVENTION STRATEGIES

Eradication therapy

The eradication of *H. pylori* infection is a cornerstone in the prevention of GC, as chronic infection is a major risk factor for the development of gastric adenocarcinoma. Current eradication therapies typically involve a combination of antibiotics (such as clarithromycin, amoxicillin, or metronidazole) and proton pump inhibitors. While these regimens have shown success in reducing *H. pylori* colonization and associated inflammation, their effectiveness in preventing GC varies depending on factors such as the timing of intervention, antibiotic resistance, and the extent of pre-existing gastric damage. Studies have demonstrated that eradication therapy is most effective in preventing GC when administered before the onset of precancerous lesions, such as atrophic gastritis or intestinal metaplasia. However, in advanced stages of gastric carcinogenesis, eradication alone may not be sufficient to reverse the damage, highlighting the need for early detection and treatment of *H. pylori* infection[77,78].

Several key randomized controlled trials (RCTs) have shown that *H. pylori* eradication therapy is most effective in preventing GC when administered before the development of precancerous lesions such as atrophic gastritis or intestinal metaplasia. In the Shandong intervention trial[79], a double-blind, factorial RCT with over 3000 participants evaluated the effects of antibiotics, vitamins, and garlic on precancerous lesions and found a significant reduction in lesion progression, especially when eradication was performed early. The Wong *et al*[80] study randomized 1630 individuals in a high-risk Chinese population to receive *H. pylori* treatment or placebo and demonstrated a reduced incidence of GC primarily in those without baseline intestinal metaplasia or dysplasia. Similarly, Ma *et al*[81] followed a Chinese cohort over 15 years and found that early eradication significantly reduced GC incidence, particularly in participants without precancerous changes. Finally, the Fukase *et al*[82] trial, conducted in Japanese patients with early GC post-endoscopic resection, showed that eradication reduced the risk of metachronous GC by 66%. Across all studies, the primary outcome was the incidence or progression of GC, and the study designs were robust, long-term RCTs with histological or clinical endpoints to assess cancer risk.

Precancerous gastric lesions, including atrophic gastritis and intestinal metaplasia, are assessed histologically using validated staging systems. The Operative Link on Gastritis Assessment (OLGA) system, as described by Rugge *et al*[83], quantifies the severity and topographic extent of gastric atrophy by scoring biopsies from the antrum and corpus. This staging correlates well with GC risk. Capelle *et al*[84] further validated that intestinal metaplasia, when used in place of atrophy, provides equally reliable staging within the OLGA framework.

In large trials like the Shandong Intervention Trial by You *et al*[79] and the 15-year follow-up study by Ma *et al*[81], gastric mucosal biopsies were evaluated using the Updated Sydney System, which semiquantitatively scores atrophy and intestinal metaplasia (none, mild, moderate, marked) across multiple gastric sites. These studies demonstrated that eradication of *H. pylori* before the onset or in early stages of these lesions significantly reduces progression and the risk of GC.

Several studies and meta-analyses have explored whether different antibiotic regimens for *H. pylori* eradication vary in their GC prevention efficacy. While Ma *et al*[81] demonstrated that *H. pylori* eradication (regardless of regimen) significantly reduces GC incidence over 15 years, Liou *et al*[85] provided evidence that bismuth quadruple and concomitant therapies achieved significantly higher eradication rates than standard triple therapy. Although GC outcomes were not directly compared across regimens in Liou *et al*'s study, improved eradication correlates with better cancer prevention[85]. Lee *et al*[86] confirmed through meta-analysis that *H. pylori* eradication significantly lowers GC risk, though regimen-specific comparisons were limited. The American College of Gastroenterologists guidelines[87] recommend tailored therapy based on local antibiotic resistance, highlighting that higher eradication efficacy, particularly in resistant regions, is key to maximizing cancer prevention. While GC prevention is more dependent on successful eradication than the specific regimen, comparative studies have shown that newer regimens (bismuth quadruple, concomitant) yield significantly better eradication rates, especially in areas with high resistance, indirectly improving cancer prevention outcomes.

Antibiotic resistance, particularly to clarithromycin, is a major factor reducing the success rate of *H. pylori* eradication, with treatment failure occurring in areas where resistance exceeds 15%[87,88]. The Maastricht VI/Florence consensus recommends avoiding standard triple therapy in high-resistance regions and favoring regimens like bismuth quadruple or concomitant therapy. To mitigate resistance-related failure, strategies include tailored therapy based on susceptibility testing and adopting novel acid suppression approaches, such as vonoprazan-based dual therapy, which demonstrated high eradication rates even in resistant strains[89].

Post-*H. pylori* eradication, the gastric mucosa experiences a significant reduction in inflammatory mediators, oxidative stress, epithelial proliferation, and microbial imbalance, all of which lower the risk of GC, especially when intervention occurs before advanced precancerous changes develop. Following *H. pylori* eradication, inflammatory markers and microbial dysbiosis in the gastric microenvironment are significantly reduced, which contributes to lowering GC risk. Schulz *et al*[90] showed that eradication restores a healthier gastric microbiota composition, reducing pro-inflammatory bacterial species. Nakajima *et al*[91] demonstrated that eradication can partially reverse epigenetic alterations, such as CDH1 gene methylation, which are key drivers in gastric carcinogenesis. Additionally, Tahara[92] highlighted that persistent inflammation promotes oxidative DNA damage and mutagenesis; thus, resolving inflammation post-eradication helps stabilize the mucosa and suppress neoplastic transformation.

Evidence suggests that *H. pylori* eradication can still provide prognostic benefit and partially reverse gastric mucosal damage even in advanced stages of gastric carcinogenesis. According to Wang *et al*[93], eradication significantly improves gastric atrophy and shows limited but possible regression of intestinal metaplasia, especially in less extensive cases. Shin *et al*[94] demonstrated that eradication reduces aberrant DNA methylation over a 10-year period, suggesting reversal of molecular damage associated with cancer progression. Additionally, Choi *et al*[95] found that eradication therapy significantly lowers the risk of metachronous GC after endoscopic resection of early GC, highlighting its role in improving long-term outcomes even when precancerous changes are already present.

Vaccine development

Vaccine development represents a promising strategy for the primary prevention of *H. pylori*-induced GC. Research has focused on identifying immunogenic antigens, such as urease, VacA, and CagA, which play critical roles in *H. pylori* pathogenesis. Advances in vaccine technology, including the use of recombinant proteins, DNA vaccines, and mucosal delivery systems, have shown potential in eliciting robust immune responses in preclinical models. However, challenges remain in translating these findings into effective human vaccines, including the need for long-lasting immunity and the ability to overcome the bacterium's immune evasion strategies. Despite these hurdles, ongoing clinical trials and innovative approaches, such as multi-epitope vaccines and adjuvants, continue to drive progress in this field[96-99].

Targeted molecular therapy

The molecular mechanisms underlying *H. pylori*-induced carcinogenesis provide opportunities for targeted therapies aimed at disrupting specific pathways involved in GC development. Key pathways include the NF-κB and MAPK signaling cascades, which are activated by *H. pylori* virulence factors such as CagA and promote inflammation and cell proliferation. Additionally, targeting oxidative stress and DNA damage responses, which are exacerbated by chronic infection, may help mitigate carcinogenic processes. Small molecule inhibitors, monoclonal antibodies, and epigenetic modulators are being explored as potential therapeutic agents to block these pathways. Combining targeted molecular therapies with eradication regimens or vaccines could enhance the prevention and treatment of *H. pylori*-associated GC, offering a multifaceted approach to reducing the global burden of this disease[96].

Understanding *H. Pylori*'s molecular mechanisms can pave the way for targeted therapies and preventive strategies by pinpointing key virulence factors, host-pathogen interactions, and immune evasion strategies. For example, targeting toxins like CagA and VacA, which disrupt host cell signaling and promote inflammation and cancer, could involve developing inhibitors that block their secretion through the T4SS or neutralizing their effects with monoclonal antibodies. Additionally, since *H. pylori* relies on urease to survive stomach acid, small-molecule urease inhibitors could jeopardise its colonization. Disrupting bacterial adhesion molecules like BabA and SabA, could prevent infection using synthetic analogs that competitively block these interactions. Furthermore, since *H. pylori* forms biofilms that enhance antibiotic resistance, quorum-sensing inhibitors or biofilm-disrupting agents could improve treatment efficacy. On the host side, modulating immune responses, such as balancing Treg/Th17 activity or manipulating TLR/NLR signaling, could reduce chronic inflammation without eliminating protective immunity. Meanwhile, vaccine development using CagA, VacA, or urease subunits, along with engineered probiotics that competitively exclude *H. pylori*, could offer preventive solutions. Finally, personalized approaches, such as screening for high-risk strains (CagA+/VacA+) or detecting early molecular biomarkers, could enable preemptive treatment before malignancy develops. By leveraging these molecular insights, researchers can design more effective, tailored interventions to combat *H. pylori* infections and their devastating consequences, including GC[100-102].

CONCLUSION

In conclusion, *H. pylori* infection represents a significant risk factor for a spectrum of gastroduodenal pathologies, encompassing PUs and gastric adenocarcinoma. This bacterium employs a multifaceted pathogenesis strategy. *H. pylori* utilizes urease activity to neutralize gastric acid, facilitating colonization. Flagella enable motility towards the epithelium, where adherence occurs *via* bacterial adhesins. Virulence factors like CagA disrupt cellular homeostasis and trigger chronic inflammation. Furthermore, *H. pylori* infection can compromise DNA repair mechanisms, promoting

mutagenesis. Host genetic polymorphisms also influence disease susceptibility. Elucidating these intricate mechanisms underlying *H. pylori* pathogenesis is paramount for developing targeted therapies to eradicate the infection and prevent its associated gastric diseases.

FOOTNOTES

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REFERENCES

1 **Park JY**, Forman D, Waskito LA, Yamaoka Y, Crabtree JE. Epidemiology of Helicobacter pylori and CagA-Positive Infections and Global Variations in Gastric Cancer. *Toxins (Basel)* 2018; **10** [*RCA*] [PMID: 29671784 DOI: 10.3390/toxins10040163] [FullText] [Full Text(PDF)]
2 **Leja M**, Grinberga-Derica I, Bilgilier C, Steininger C. Review: Epidemiology of Helicobacter pylori infection. *Helicobacter* 2019; **24** Suppl 1: e12635 [*RCA*] [PMID: 31486242 DOI: 10.1111/hel.12635] [FullText]
3 **Kotilea K**, Bontems P, Touati E. Epidemiology, Diagnosis and Risk Factors of Helicobacter pylori Infection. *Adv Exp Med Biol* 2019; **1149**: 17-33 [*RCA*] [PMID: 31016621 DOI: 10.1007/5584_2019_357] [FullText]
4 **Watari J**, Chen N, Amenta PS, Fukui H, Oshima T, Tomita T, Miwa H, Lim KJ, Das KM. Helicobacter pylori associated chronic gastritis, clinical syndromes, precancerous lesions, and pathogenesis of gastric cancer development. *World J Gastroenterol* 2014; **20**: 5461-5473 [*RCA*] [PMID: 24833876 DOI: 10.3748/wjg.v20.i18.5461] [FullText] [Full Text(PDF)]
5 **Duan Y**, Xu Y, Dou Y, Xu D. Helicobacter pylori and gastric cancer: mechanisms and new perspectives. *J Hematol Oncol* 2025; **18**: 10 [*RCA*] [PMID: 39849657 DOI: 10.1186/s13045-024-01654-2] [FullText]
6 **Jang BG**, Kim WH. Molecular pathology of gastric carcinoma. *Pathobiology* 2011; **78**: 302-310 [*RCA*] [PMID: 22104201 DOI: 10.1159/000321703] [FullText]
7 **Roberts SE**, Morrison-Rees S, Samuel DG, Thorne K, Akbari A, Williams JG. Review article: the prevalence of Helicobacter pylori and the incidence of gastric cancer across Europe. *Aliment Pharmacol Ther* 2016; **43**: 334-345 [*RCA*] [PMID: 26592801 DOI: 10.1111/apt.13474] [FullText]
8 **Zhang RG**, Duan GC, Fan QT, Chen SY. Role of Helicobacter pylori infection in pathogenesis of gastric carcinoma. *World J Gastrointest Pathophysiol* 2016; **7**: 97-107 [*RCA*] [PMID: 26909232 DOI: 10.4291/wjgp.v7.i1.97] [FullText] [Full Text(PDF)]
9 **De Falco M**, Lucariello A, Iaquinto S, Esposito V, Guerra G, De Luca A. Molecular Mechanisms of Helicobacter pylori Pathogenesis. *J Cell Physiol* 2015; **230**: 1702-1707 [*RCA*] [PMID: 25639461 DOI: 10.1002/jcp.24933] [FullText]
10 **Valenzuela MA**, Canales J, Corvalán AH, Quest AF. Helicobacter pylori-induced inflammation and epigenetic changes during gastric carcinogenesis. *World J Gastroenterol* 2015; **21**: 12742-12756 [*RCA*] [PMID: 26668499 DOI: 10.3748/wjg.v21.i45.12742] [FullText] [Full Text(PDF)]
11 **Yong X**, Tang B, Li BS, Xie R, Hu CJ, Luo G, Qin Y, Dong H, Yang SM. Helicobacter pylori virulence factor CagA promotes tumorigenesis of gastric cancer *via* multiple signaling pathways. *Cell Commun Signal* 2015; **13**: 30 [*RCA*] [PMID: 26160167 DOI: 10.1186/s12964-015-0111-0] [FullText] [Full Text(PDF)]
12 **Backert S**, Linz B, Tegtmeyer N. Helicobacter pylori-Induced Host Cell DNA Damage and Genetics of Gastric Cancer Development. *Curr Top Microbiol Immunol* 2023; **444**: 185-206 [*RCA*] [PMID: 38231219 DOI: 10.1007/978-3-031-47331-9_7] [FullText]
13 **Murata-Kamiya N**, Hatakeyama M. Helicobacter pylori-induced DNA double-stranded break in the development of gastric cancer. *Cancer Sci* 2022; **113**: 1909-1918 [*RCA*] [PMID: 35359025 DOI: 10.1111/cas.15357] [FullText] [Full Text(PDF)]
14 **Santos JC**, Ribeiro ML. Epigenetic regulation of DNA repair machinery in Helicobacter pylori-induced gastric carcinogenesis. *World J Gastroenterol* 2015; **21**: 9021-9037 [*RCA*] [PMID: 26290630 DOI: 10.3748/wjg.v21.i30.9021] [FullText] [Full Text(PDF)]
15 **Nishizawa T**, Suzuki H. Gastric Carcinogenesis and Underlying Molecular Mechanisms: Helicobacter pylori and Novel Targeted Therapy. *Biomed Res Int* 2015; **2015**: 794378 [*RCA*] [PMID: 25945346 DOI: 10.1155/2015/794378] [FullText] [Full Text(PDF)]
16 **Alipour M**. Molecular Mechanism of Helicobacter pylori-Induced Gastric Cancer. *J Gastrointest Cancer* 2021; **52**: 23-30 [*RCA*] [PMID: 32926335 DOI: 10.1007/s12029-020-00518-5] [FullText] [Full Text(PDF)]

17 **Wroblewski LE**, Peek RM Jr. Clinical Pathogenesis, Molecular Mechanisms of Gastric Cancer Development. *Curr Top Microbiol Immunol* 2023; **444**: 25-52 [*RCA*] [PMID: 38231214 DOI: 10.1007/978-3-031-47331-9_2] [FullText]

18 **Arksey H**, O'malley L. Scoping studies: towards a methodological framework. *Int J Soc Res Methodol* 2005; **8**: 19-32 [DOI: 10.1080/1364557032000119616] [FullText]

19 **Ji X**, He G, Wang K, Zhang Y, Yin J, Wang K. Estimation of gastric cancer burden attributable to Helicobacter pylori infection in Asia. *J Public Health (Oxf)* 2023; **45**: 40-46 [*RCA*] [PMID: 35137200 DOI: 10.1093/pubmed/fdab410] [FullText]

20 **Kharel S**, Bist A, Shrestha S, Homagain S. Helicobacter pylori healthy South Asians. *JGH Open* 2020; **4**: 1037-1046 [*RCA*] [PMID: 33319035 DOI: 10.1002/jgh3.12426] [FullText] [Full Text(PDF)]

21 **Sharndama HC**, Mba IE. Helicobacter pylori: an up-to-date overview on the virulence and pathogenesis mechanisms. *Braz J Microbiol* 2022; **53**: 33-50 [*RCA*] [PMID: 34988937 DOI: 10.1007/s42770-021-00675-0] [FullText] [Full Text(PDF)]

22 **Salvatori S**, Marafini I, Laudisi F, Monteleone G, Stolfi C. Helicobacter pylori and Gastric Cancer: Pathogenetic Mechanisms. *Int J Mol Sci* 2023; **24** [*RCA*] [PMID: 36769214 DOI: 10.3390/ijms24032895] [FullText] [Full Text(PDF)]

23 **Baj J**, Forma A, Sitarz M, Portincasa P, Garruti G, Krasowska D, Maciejewski R. Helicobacter pylori Virulence Factors-Mechanisms of Bacterial Pathogenicity in the Gastric Microenvironment. *Cells* 2020; **10** [*RCA*] [PMID: 33375694 DOI: 10.3390/cells10010027] [FullText] [Full Text(PDF)]

24 **Camilo V**, Sugiyama T, Touati E. Pathogenesis of Helicobacter pylori infection. *Helicobacter* 2017; **22** Suppl 1 [*RCA*] [PMID: 28891130 DOI: 10.1111/hel.12405] [FullText]

25 **Ali A**, AlHussaini KI. Helicobacter pylori: A Contemporary Perspective on Pathogenesis, Diagnosis and Treatment Strategies. *Microorganisms* 2024; **12** [*RCA*] [PMID: 38276207 DOI: 10.3390/microorganisms12010222] [FullText]

26 **Bhattacharjee A**, Sahoo OS, Sarkar A, Bhattacharya S, Chowdhury R, Kar S, Mukherjee O. Infiltration to infection: key virulence players of Helicobacter pylori pathogenicity. *Infection* 2024; **52**: 345-384 [*RCA*] [PMID: 38270780 DOI: 10.1007/s15010-023-02159-9] [FullText]

27 **Kao CY**, Sheu BS, Wu JJ. Helicobacter pylori infection: An overview of bacterial virulence factors and pathogenesis. *Biomed J* 2016; **39**: 14-23 [*RCA*] [PMID: 27105595 DOI: 10.1016/j.bj.2015.06.002] [FullText] [Full Text(PDF)]

28 **Cao L**, Zhu S, Lu H, Soutto M, Bhat N, Chen Z, Peng D, Lin J, Lu J, Li P, Zheng C, Huang C, El-Rifai W. Helicobacter pylori-induced RASAL2 Through Activation of Nuclear Factor-κB Promotes Gastric Tumorigenesis *via* β-catenin Signaling Axis. *Gastroenterology* 2022; **162**: 1716-1731.e17 [*RCA*] [PMID: 35134322 DOI: 10.1053/j.gastro.2022.01.046] [FullText]

29 **Imai S**, Ooki T, Murata-Kamiya N, Komura D, Tahmina K, Wu W, Takahashi-Kanemitsu A, Knight CT, Kunita A, Suzuki N, Del Valle AA, Tsuboi M, Hata M, Hayakawa Y, Ohnishi N, Ueda K, Fukayama M, Ushiku T, Ishikawa S, Hatakeyama M. Helicobacter pylori CagA elicits BRCAness to induce genome instability that may underlie bacterial gastric carcinogenesis. *Cell Host Microbe* 2021; **29**: 941-958.e10 [*RCA*] [PMID: 33989515 DOI: 10.1016/j.chom.2021.04.006] [FullText]

30 **Link A**, Bornschein J, Thon C. Helicobacter pylori induced gastric carcinogenesis - The best molecular model we have? *Best Pract Res Clin Gastroenterol* 2021; **50-51**: 101743 [*RCA*] [PMID: 33975683 DOI: 10.1016/j.bpg.2021.101743] [FullText]

31 **Chen B**, Liu X, Yu P, Xie F, Kwan JSH, Chan WN, Fang C, Zhang J, Cheung AHK, Chow C, Leung GWM, Leung KT, Shi S, Zhang B, Wang S, Xu D, Fu K, Wong CC, Wu WKK, Chan MWY, Tang PMK, Tsang CM, Lo KW, Tse GMK, Yu J, To KF, Kang W. H. pylori-induced NF-κB-PIEZO1-YAP1-CTGF axis drives gastric cancer progression and cancer-associated fibroblast-mediated tumour microenvironment remodelling. *Clin Transl Med* 2023; **13**: e1481 [*RCA*] [PMID: 37983931 DOI: 10.1002/ctm2.1481] [FullText] [Full Text(PDF)]

32 **Chen SY**, Zhang RG, Duan GC. Pathogenic mechanisms of the oncoprotein CagA in H. pylori-induced gastric cancer (Review). *Oncol Rep* 2016; **36**: 3087-3094 [*RCA*] [PMID: 27748858 DOI: 10.3892/or.2016.5145] [FullText]

33 **Liu D**, Liu Y, Zhu W, Lu Y, Zhu J, Ma X, Xing Y, Yuan M, Ning B, Wang Y, Jia Y. Helicobacter pylori-induced aberrant demethylation and expression of GNB4 promotes gastric carcinogenesis *via* the Hippo-YAP1 pathway. *BMC Med* 2023; **21**: 134 [*RCA*] [PMID: 37016382 DOI: 10.1186/s12916-023-02842-6] [FullText]

34 **Li N**, Feng Y, Hu Y, He C, Xie C, Ouyang Y, Artim SC, Huang D, Zhu Y, Luo Z, Ge Z, Lu N. Helicobacter pylori CagA promotes epithelial mesenchymal transition in gastric carcinogenesis *via* triggering oncogenic YAP pathway. *J Exp Clin Cancer Res* 2018; **37**: 280 [*RCA*] [PMID: 30466467 DOI: 10.1186/s13046-018-0962-5] [FullText] [Full Text(PDF)]

35 **Liu Y**, Zhang B, Zhou Y, Xing Y, Wang Y, Jia Y, Liu D. Targeting Hippo pathway: A novel strategy for Helicobacter pylori-induced gastric cancer treatment. *Biomed Pharmacother* 2023; **161**: 114549 [*RCA*] [PMID: 36958190 DOI: 10.1016/j.biopha.2023.114549] [FullText]

36 **Kubo S**, Ninomiya R, Kajiwara T, Tokunaga A, Matsuda S, Murakami K, Yamaoka Y, Aigaki T, Hamada F. Helicobacter pylori virulence factor CagA promotes Snail-mediated epithelial-mesenchymal transition and invasive behavior by downregulating Semaphorin 5A in gastric epithelial cells. *Biochem Biophys Res Commun* 2025; **750**: 151421 [*RCA*] [PMID: 39892055 DOI: 10.1016/j.bbrc.2025.151421] [FullText]

37 **Sougleri IS**, Papadakos KS, Zadik MP, Mavri-Vavagianni M, Mentis AF, Sgouras DN. Helicobacter pylori CagA protein induces factors involved in the epithelial to mesenchymal transition (EMT) in infected gastric epithelial cells in an EPIYA- phosphorylation-dependent manner. *FEBS J* 2016; **283**: 206-220 [*RCA*] [PMID: 26907789 DOI: 10.1111/febs.13592] [FullText]

38 **Yu H**, Zeng J, Liang X, Wang W, Zhou Y, Sun Y, Liu S, Li W, Chen C, Jia J. Helicobacter pylori promotes epithelial-mesenchymal transition in gastric cancer by downregulating programmed cell death protein 4 (PDCD4). *PLoS One* 2014; **9**: e105306 [*RCA*] [PMID: 25144746 DOI: 10.1371/journal.pone.0105306] [FullText] [Full Text(PDF)]

39 **Messina B**, Lo Sardo F, Scalera S, Memeo L, Colarossi C, Mare M, Blandino G, Ciliberto G, Maugeri-Saccà M, Bon G. Hippo pathway dysregulation in gastric cancer: from Helicobacter pylori infection to tumor promotion and progression. *Cell Death Dis* 2023; **14**: 21 [*RCA*] [PMID: 36635265 DOI: 10.1038/s41419-023-05568-8] [FullText]

40 **Akazawa Y**, Isomoto H, Matsushima K, Kanda T, Minami H, Yamaghchi N, Taura N, Shiozawa K, Ohnita K, Takeshima F, Nakano M, Moss J, Hirayama T, Nakao K. Endoplasmic reticulum stress contributes to Helicobacter pylori VacA-induced apoptosis. *PLoS One* 2013; **8**: e82322 [*RCA*] [PMID: 24349255 DOI: 10.1371/journal.pone.0082322] [FullText] [Full Text(PDF)]

41 **Mi Y**, Dong H, Sun X, Ren F, Tang Y, Zheng P. The association of Helicobacter pylori CagA EPIYA motifs and vacA genotypes with homologous recombination repair markers during the gastric precancerous cascade. *Int J Biol Markers* 2020; **35**: 49-55 [*RCA*] [PMID: 32286927 DOI: 10.1177/1724600820914935] [FullText]

42 **Wessler S**, Krisch LM, Elmer DP, Aberger F. From inflammation to gastric cancer - the importance of Hedgehog/GLI signaling in Helicobacter pylori-induced chronic inflammatory and neoplastic diseases. *Cell Commun Signal* 2017; **15**: 15 [*RCA*] [PMID: 28427431 DOI: 10.1186/s12964-017-0171-4] [FullText] [Full Text(PDF)]

43 **Qadri Q**, Rasool R, Gulzar GM, Naqash S, Shah ZA. H. pylori infection, inflammation and gastric cancer. *J Gastrointest Cancer* 2014; **45**:

126-132 [*RCA*] [PMID: 24557546 DOI: 10.1007/s12029-014-9583-1] [FullText]

44 **Wang F**, Meng W, Wang B, Qiao L. Helicobacter pylori-induced gastric inflammation and gastric cancer. *Cancer Lett* 2014; **345**: 196-202 [*RCA*] [PMID: 23981572 DOI: 10.1016/j.canlet.2013.08.016] [FullText]

45 **Sah DK**, Arjunan A, Lee B, Jung YD. Reactive Oxygen Species and H. pylori Infection: A Comprehensive Review of Their Roles in Gastric Cancer Development. *Antioxidants (Basel)* 2023; **12** [*RCA*] [PMID: 37760015 DOI: 10.3390/antiox12091712] [FullText]

46 **Gu Y**, Xu Y, Wang P, Zhao Y, Wan C. Research progress on molecular mechanism of pyroptosis caused by Helicobacter pylori in gastric cancer. *Ann Med Surg (Lond)* 2024; **86**: 2016-2022 [*RCA*] [PMID: 38576917 DOI: 10.1097/MS9.0000000000001802] [FullText] [Full Text (PDF)]

47 **Wu S**, Chen Y, Chen Z, Wei F, Zhou Q, Li P, Gu Q. Reactive oxygen species and gastric carcinogenesis: The complex interaction between Helicobacter pylori and host. *Helicobacter* 2023; **28**: e13024 [*RCA*] [PMID: 37798959 DOI: 10.1111/hel.13024] [FullText]

48 **Jain U**, Saxena K, Chauhan N. Helicobacter pylori induced reactive oxygen Species: A new and developing platform for detection. *Helicobacter* 2021; **26**: e12796 [*RCA*] [PMID: 33666321 DOI: 10.1111/hel.12796] [FullText]

49 **Raza Y**, Khan A, Farooqui A, Mubarak M, Facista A, Akhtar SS, Khan S, Kazi JI, Bernstein C, Kazmi SU. Oxidative DNA damage as a potential early biomarker of Helicobacter pylori associated carcinogenesis. *Pathol Oncol Res* 2014; **20**: 839-846 [*RCA*] [PMID: 24664859 DOI: 10.1007/s12253-014-9762-1] [FullText]

50 **Fu HW**, Lai YC. The Role of Helicobacter pylori Neutrophil-Activating Protein in the Pathogenesis of H. pylori and Beyond: From a Virulence Factor to Therapeutic Targets and Therapeutic Agents. *Int J Mol Sci* 2022; **24** [*RCA*] [PMID: 36613542 DOI: 10.3390/ijms24010091] [FullText] [Full Text(PDF)]

51 **Faass L**, Hauke M, Stein SC, Josenhans C. Innate activation of human neutrophils and neutrophil-like cells by the pro-inflammatory bacterial metabolite ADP-heptose and Helicobacter pylori. *Int J Med Microbiol* 2023; **313**: 151585 [*RCA*] [PMID: 37399704 DOI: 10.1016/j.ijmm.2023.151585] [FullText]

52 **Faass L**, Hauke M, Stein SC, Josenhans C. Innate immune activation and modulatory factors of Helicobacter pylori towards phagocytic and nonphagocytic cells. *Curr Opin Immunol* 2023; **82**: 102301 [*RCA*] [PMID: 36933362 DOI: 10.1016/j.coi.2023.102301] [FullText]

53 **Katsurahara M**, Kobayashi Y, Iwasa M, Ma N, Inoue H, Fujita N, Tanaka K, Horiki N, Gabazza EC, Takei Y. Reactive nitrogen species mediate DNA damage in Helicobacter pylori-infected gastric mucosa. *Helicobacter* 2009; **14**: 552-558 [*RCA*] [PMID: 19889073 DOI: 10.1111/j.1523-5378.2009.00719.x] [FullText]

54 **Ding SZ**, O'Hara AM, Denning TL, Dirden-Kramer B, Mifflin RC, Reyes VE, Ryan KA, Elliott SN, Izumi T, Boldogh I, Mitra S, Ernst PB, Crowe SE. Helicobacter pylori and H2O2 increase AP endonuclease-1/redox factor-1 expression in human gastric epithelial cells. *Gastroenterology* 2004; **127**: 845-858 [*RCA*] [PMID: 15362040 DOI: 10.1053/j.gastro.2004.06.017] [FullText]

55 **Bhattacharyya A**, Chattopadhyay R, Burnette BR, Cross JV, Mitra S, Ernst PB, Bhakat KK, Crowe SE. Acetylation of apurinic/apyrimidinic endonuclease-1 regulates Helicobacter pylori-mediated gastric epithelial cell apoptosis. *Gastroenterology* 2009; **136**: 2258-2269 [*RCA*] [PMID: 19505426 DOI: 10.1053/j.gastro.2009.02.014] [FullText] [Full Text(PDF)]

56 **Caruso R**, Pallone F, Monteleone G. Emerging role of IL-23/IL-17 axis in H pylori-associated pathology. *World J Gastroenterol* 2007; **13**: 5547-5551 [*RCA*] [PMID: 17948927 DOI: 10.3748/wjg.v13.i42.5547] [FullText] [Full Text(PDF)]

57 **Dewayani A**, Fauzia KA, Alfaray RI, Waskito LA, Doohan D, Rezkitha YAA, Abdurachman A, Kobayashi T, I'tishom R, Yamaoka Y, Miftahussurur M. The Roles of IL-17, IL-21, and IL-23 in the Helicobacter pylori Infection and Gastrointestinal Inflammation: A Review. *Toxins (Basel)* 2021; **13** [*RCA*] [PMID: 33924897 DOI: 10.3390/toxins13050315] [FullText] [Full Text(PDF)]

58 **Bagheri N**, Salimzadeh L, Shirzad H. The role of T helper 1-cell response in Helicobacter pylori-infection. *Microb Pathog* 2018; **123**: 1-8 [*RCA*] [PMID: 29936093 DOI: 10.1016/j.micpath.2018.06.033] [FullText]

59 **D'Elios MM**, Manghetti M, De Carli M, Costa F, Baldari CT, Burroni D, Telford JL, Romagnani S, Del Prete G. T helper 1 effector cells specific for Helicobacter pylori in the gastric antrum of patients with peptic ulcer disease. *J Immunol* 1997; **158**: 962-967 [*RCA*] [PMID: 8993017] [FullText]

60 **D'Elios MM**, Manghetti M, Almerigogna F, Amedei A, Costa F, Burroni D, Baldari CT, Romagnani S, Telford JL, Del Prete G. Different cytokine profile and antigen-specificity repertoire in Helicobacter pylori-specific T cell clones from the antrum of chronic gastritis patients with or without peptic ulcer. *Eur J Immunol* 1997; **27**: 1751-1755 [*RCA*] [PMID: 9247587 DOI: 10.1002/eji.1830270723] [FullText]

61 **Morey P**, Pfannkuch L, Pang E, Boccellato F, Sigal M, Imai-Matsushima A, Dyer V, Koch M, Mollenkopf HJ, Schlaermann P, Meyer TF. Helicobacter pylori Depletes Cholesterol in Gastric Glands to Prevent Interferon Gamma Signaling and Escape the Inflammatory Response. *Gastroenterology* 2018; **154**: 1391-1404.e9 [*RCA*] [PMID: 29273450 DOI: 10.1053/j.gastro.2017.12.008] [FullText]

62 **Clyne M**, Rowland M. The Role of Host Genetic Polymorphisms in Helicobacter pylori Mediated Disease Outcome. *Adv Exp Med Biol* 2019; **1149**: 151-172 [*RCA*] [PMID: 31016623 DOI: 10.1007/5584_2019_364] [FullText]

63 **Maria de Oliveira Barboza M**, Ferreira da Costa R, Paulo Por Deus Gomes J, Mário Rodríguez Burbano R, Goberlânio de Barros Silva P, Helena Barem Rabenhorst S. Host repair polymorphisms and H. pylori genes in gastric disease outcomes: Who are the guardian and villains? *Gene* 2025; **933**: 148977 [*RCA*] [PMID: 39389328 DOI: 10.1016/j.gene.2024.148977] [FullText]

64 **Ladeira MS**, Rodrigues MA, Salvadori DM, Queiroz DM, Freire-Maia DV. DNA damage in patients infected by Helicobacter pylori. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 631-637 [*RCA*] [PMID: 15066929] [FullText]

65 **Hardbower DM**, Peek RM Jr, Wilson KT. At the Bench: Helicobacter pylori, dysregulated host responses, DNA damage, and gastric cancer. *J Leukoc Biol* 2014; **96**: 201-212 [*RCA*] [PMID: 24868089 DOI: 10.1189/jlb.4BT0214-099R] [FullText]

66 **Toller IM**, Neelsen KJ, Steger M, Hartung ML, Hottiger MO, Stucki M, Kalali B, Gerhard M, Sartori AA, Lopes M, Müller A. Carcinogenic bacterial pathogen Helicobacter pylori triggers DNA double-strand breaks and a DNA damage response in its host cells. *Proc Natl Acad Sci U S A* 2011; **108**: 14944-14949 [*RCA*] [PMID: 21896770 DOI: 10.1073/pnas.1100959108] [FullText]

67 **Raza Y**, Ahmed A, Khan A, Chishti AA, Akhter SS, Mubarak M, Bernstein C, Zaitlin B, Kazmi SU. Helicobacter pylori severely reduces expression of DNA repair proteins PMS2 and ERCC1 in gastritis and gastric cancer. *DNA Repair (Amst)* 2020; **89**: 102836 [*RCA*] [PMID: 32143126 DOI: 10.1016/j.dnarep.2020.102836] [FullText]

68 **Park DI**, Park SH, Kim SH, Kim JW, Cho YK, Kim HJ, Sohn CI, Jeon WK, Kim BI, Cho EY, Kim EJ, Chae SW, Sohn JH, Sung IK, Sepulveda AR, Kim JJ. Effect of Helicobacter pylori infection on the expression of DNA mismatch repair protein. *Helicobacter* 2005; **10**: 179-184 [*RCA*] [PMID: 15904475 DOI: 10.1111/j.1523-5378.2005.00309.x] [FullText]

69 **Bartchewsky W Jr**, Martini MR, Squassoni AC, Alvarez MC, Ladeira MS, Salvatore DM, Trevisan MA, Pedrazzoli J Jr, Ribeiro ML. Influence of Helicobacter pylori infection on the expression of MLH1 and MGMT in patients with chronic gastritis and gastric cancer. *Eur J*

Clin Microbiol Infect Dis 2009; **28**: 591-597 [*RCA*] [PMID: 19089477 DOI: 10.1007/s10096-008-0676-2] [FullText]

70 **Ooki A**, Osumi H, Yoshino K, Yamaguchi K. Potent therapeutic strategy in gastric cancer with microsatellite instability-high and/or deficient mismatch repair. *Gastric Cancer* 2024; **27**: 907-931 [*RCA*] [PMID: 38922524 DOI: 10.1007/s10120-024-01523-4] [FullText]

71 **Yao Y**, Tao H, Kim JJ, Burkhead B, Carloni E, Gasbarrini A, Sepulveda AR. Alterations of DNA mismatch repair proteins and microsatellite instability levels in gastric cancer cell lines. *Lab Invest* 2004; **84**: 915-922 [*RCA*] [PMID: 15133479 DOI: 10.1038/labinvest.3700117] [Full Text]

72 **Shimizu T**, Marusawa H, Watanabe N, Chiba T. Molecular Pathogenesis of Helicobacter pylori-Related Gastric Cancer. *Gastroenterol Clin North Am* 2015; **44**: 625-638 [*RCA*] [PMID: 26314672 DOI: 10.1016/j.gtc.2015.05.011] [FullText]

73 **Assumpção PP**, Genta RM, Camargo MC, Silva JMCD, Amieva MR, Rugge M. The Landscape of Helicobacter pylori-related Gastric Carcinogenesis. *J Gastrointestin Liver Dis* 2024; **33**: 524-534 [*RCA*] [PMID: 39733312 DOI: 10.15403/jgld-5959] [FullText]

74 **Hess T**, Maj C, Gehlen J, Borisov O, Haas SL, Gockel I, Vieth M, Piessen G, Alakus H, Vashist Y, Pereira C, Knapp M, Schüller V, Quaas A, Grabsch HI, Trautmann J, Malecka-Wojciesko E, Mokrowiecka A, Speller J, Mayr A, Schröder J, Hillmer AM, Heider D, Lordick F, Pérez-Aísa Á, Campo R, Espinel J, Geijo F, Thomson C, Bujanda L, Sopeña F, Lanas Á, Pellisé M, Pauligk C, Goetze TO, Zelck C, Reingruber J, Hassanin E, Elbe P, Alsabeah S, Lindblad M, Nilsson M, Kreuser N, Thieme R, Tavano F, Pastorino R, Arzani D, Persiani R, Jung JO, Nienhüser H, Ott K, Schumann RR, Kumpf O, Burock S, Arndt V, Jakubowska A, Ławniczak M, Moreno V, Martín V, Kogevinas M, Pollán M, Dąbrowska J, Salas A, Cussenot O, Boland-Auge A, Daian D, Deleuze JF, Salvi E, Teder-Laving M, Tomasello G, Ratti M, Senti C, De Re V, Steffan A, Hölscher AH, Messerle K, Bruns CJ, Sīviņš A, Bogdanova I, Skieceviciene J, Arstikyte J, Moehler M, Lang H, Grimminger PP, Kruschewski M, Vassos N, Schildberg C, Lingohr P, Ridwelski K, Lippert H, Fricker N, Krawitz P, Hoffmann P, Nöthen MM, Veits L, Izbicki JR, Mostowska A, Martinón-Torres F, Cusi D, Adolfsson R, Cancel-Tassin G, Höblinger A, Rodermann E, Ludwig M, Keller G, Metspalu A, Brenner H, Heller J, Neef M, Schepke M, Dumoulin FL, Hamann L, Cannizzaro R, Ghidini M, Plaßmann D, Geppert M, Malfertheiner P, Gehlen O, Skoczylas T, Majewski M, Lubiński J, Palmieri O, Boccia S, Latiano A, Aragones N, Schmidt T, Dinis-Ribeiro M, Medeiros R, Al-Batran SE, Leja M, Kupcinskas J, García-González MA, Venerito M, Schumacher J. Dissecting the genetic heterogeneity of gastric cancer. *EBioMedicine* 2023; **92**: 104616 [*RCA*] [PMID: 37209533 DOI: 10.1016/j.ebiom.2023.104616] [FullText] [Full Text(PDF)]

75 **Cancer Genome Atlas Research Network**. Comprehensive molecular characterization of gastric adenocarcinoma. *Nature* 2014; **513**: 202-209 [*RCA*] [PMID: 25079317 DOI: 10.1038/nature13480] [FullText] [Full Text(PDF)]

76 **Cristescu R**, Lee J, Nebozhyn M, Kim KM, Ting JC, Wong SS, Liu J, Yue YG, Wang J, Yu K, Ye XS, Do IG, Liu S, Gong L, Fu J, Jin JG, Choi MG, Sohn TS, Lee JH, Bae JM, Kim ST, Park SH, Sohn I, Jung SH, Tan P, Chen R, Hardwick J, Kang WK, Ayers M, Hongyue D, Reinhard C, Loboda A, Kim S, Aggarwal A. Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med* 2015; **21**: 449-456 [*RCA*] [PMID: 25894828 DOI: 10.1038/nm.3850] [FullText]

77 **Georgopoulos S**, Papastergiou V. An update on current and advancing pharmacotherapy options for the treatment of H. pylori infection. *Expert Opin Pharmacother* 2021; **22**: 729-741 [*RCA*] [PMID: 33131337 DOI: 10.1080/14656566.2020.1845649] [FullText]

78 **Godavarthy PK**, Puli C. From Antibiotic Resistance to Antibiotic Renaissance: A New Era in Helicobacter pylori Treatment. *Cureus* 2023; **15**: e36041 [*RCA*] [PMID: 36919111 DOI: 10.7759/cureus.36041] [FullText]

79 **You WC**, Brown LM, Zhang L, Li JY, Jin ML, Chang YS, Ma JL, Pan KF, Liu WD, Hu Y, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF Jr, Xu GW, Gail MH. Randomized double-blind factorial trial of three treatments to reduce the prevalence of precancerous gastric lesions. *J Natl Cancer Inst* 2006; **98**: 974-983 [*RCA*] [PMID: 16849680 DOI: 10.1093/jnci/djj264] [FullText]

80 **Wong BC**, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, Lai KC, Hu WH, Yuen ST, Leung SY, Fong DY, Ho J, Ching CK, Chen JS; China Gastric Cancer Study Group. Helicobacter pylori eradication to prevent gastric cancer in a high-risk region of China: a randomized controlled trial. *JAMA* 2004; **291**: 187-194 [*RCA*] [PMID: 14722144 DOI: 10.1001/jama.291.2.187] [FullText]

81 **Ma JL**, Zhang L, Brown LM, Li JY, Shen L, Pan KF, Liu WD, Hu Y, Han ZX, Crystal-Mansour S, Pee D, Blot WJ, Fraumeni JF Jr, You WC, Gail MH. Fifteen-year effects of Helicobacter pylori, garlic, and vitamin treatments on gastric cancer incidence and mortality. *J Natl Cancer Inst* 2012; **104**: 488-492 [*RCA*] [PMID: 22271764 DOI: 10.1093/jnci/djs003] [FullText]

82 **Fukase K**, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Amagai K, Hayashi S, Asaka M; Japan Gast Study Group. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. *Lancet* 2008; **372**: 392-397 [*RCA*] [PMID: 18675689 DOI: 10.1016/S0140-6736(08)61159-9] [FullText]

83 **Rugge M**, Meggio A, Pennelli G, Piscioli F, Giacomelli L, De Pretis G, Graham DY. Gastritis staging in clinical practice: the OLGA staging system. *Gut* 2007; **56**: 631-636 [*RCA*] [PMID: 17142647 DOI: 10.1136/gut.2006.106666] [FullText]

84 **Capelle LG**, de Vries AC, Haringsma J, Ter Borg F, de Vries RA, Bruno MJ, van Dekken H, Meijer J, van Grieken NC, Kuipers EJ. The staging of gastritis with the OLGA system by using intestinal metaplasia as an accurate alternative for atrophic gastritis. *Gastrointest Endosc* 2010; **71**: 1150-1158 [*RCA*] [PMID: 20381801 DOI: 10.1016/j.gie.2009.12.029] [FullText]

85 **Liou JM**, Fang YJ, Chen CC, Bair MJ, Chang CY, Lee YC, Chen MJ, Chen CC, Tseng CH, Hsu YC, Lee JY, Yang TH, Luo JC, Chang CC, Chen CY, Chen PY, Shun CT, Hsu WF, Hu WH, Chen YN, Sheu BS, Lin JT, Wu JY, El-Omar EM, Wu MS; Taiwan Gastrointestinal Disease and Helicobacter Consortium. Concomitant, bismuth quadruple, and 14-day triple therapy in the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. *Lancet* 2016; **388**: 2355-2365 [*RCA*] [PMID: 27769562 DOI: 10.1016/S0140-6736(16)31409-X] [FullText]

86 **Lee YC**, Chiang TH, Chou CK, Tu YK, Liao WC, Wu MS, Graham DY. Association Between Helicobacter pylori Eradication and Gastric Cancer Incidence: A Systematic Review and Meta-analysis. *Gastroenterology* 2016; **150**: 1113-1124.e5 [*RCA*] [PMID: 26836587 DOI: 10.1053/j.gastro.2016.01.028] [FullText]

87 **Chey WD**, Leontiadis GI, Howden CW, Moss SF. ACG Clinical Guideline: Treatment of Helicobacter pylori Infection. *Am J Gastroenterol* 2017; **112**: 212-239 [*RCA*] [PMID: 28071659 DOI: 10.1038/ajg.2016.563] [FullText]

88 **Malfertheiner P**, Megraud F, Rokkas T, Gisbert JP, Liou JM, Schulz C, Gasbarrini A, Hunt RH, Leja M, O'Morain C, Rugge M, Suerbaum S, Tilg H, Sugano K, El-Omar EM; European Helicobacter and Microbiota Study group. Management of Helicobacter pylori infection: the Maastricht VI/Florence consensus report. *Gut* 2022 [*RCA*] [PMID: 35944925 DOI: 10.1136/gutjnl-2022-327745] [FullText]

89 **Suzuki S**, Gotoda T, Kusano C, Ikehara H, Ichijima R, Ohyauchi M, Ito H, Kawamura M, Ogata Y, Ohtaka M, Nakahara M, Kawabe K. Seven-day vonoprazan and low-dose amoxicillin dual therapy as first-line Helicobacter pylori treatment: a multicentre randomised trial in Japan. *Gut* 2020; **69**: 1019-1026 [*RCA*] [PMID: 31915235 DOI: 10.1136/gutjnl-2019-319954] [FullText] [Full Text(PDF)]

90 **Schulz C**, Schütte K, Koch N, Vilchez-Vargas R, Wos-Oxley ML, Oxley APA, Vital M, Malfertheiner P, Pieper DH. The active bacterial assemblages of the upper GI tract in individuals with and without Helicobacter infection. *Gut* 2018; **67**: 216-225 [*RCA*] [PMID: 27920199 DOI: 10.1136/gutjnl-2016-312904] [FullText]

91 **Nakajima T**, Enomoto S, Yamashita S, Ando T, Nakanishi Y, Nakazawa K, Oda I, Gotoda T, Ushijima T. Persistence of a component of DNA methylation in gastric mucosae after Helicobacter pylori eradication. *J Gastroenterol* 2010; **45**: 37-44 [*RCA*] [PMID: 19821005 DOI: 10.1007/s00535-009-0142-7] [FullText]

92 **Tahara E**. Molecular mechanism of stomach carcinogenesis. *J Cancer Res Clin Oncol* 1993; **119**: 265-272 [*RCA*] [PMID: 8440743 DOI: 10.1007/BF01212724] [FullText]

93 **Wang J**, Xu L, Shi R, Huang X, Li SW, Huang Z, Zhang G. Gastric atrophy and intestinal metaplasia before and after Helicobacter pylori eradication: a meta-analysis. *Digestion* 2011; **83**: 253-260 [*RCA*] [PMID: 21282951 DOI: 10.1159/000280318] [FullText]

94 **Shin CM**, Kim N, Lee HS, Park JH, Ahn S, Kang GH, Kim JM, Kim JS, Lee DH, Jung HC. Changes in aberrant DNA methylation after Helicobacter pylori eradication: a long-term follow-up study. *Int J Cancer* 2013; **133**: 2034-2042 [*RCA*] [PMID: 23595635 DOI: 10.1002/ijc.28219] [FullText]

95 **Choi IJ**, Kook MC, Kim YI, Cho SJ, Lee JY, Kim CG, Park B, Nam BH. Helicobacter pylori Therapy for the Prevention of Metachronous Gastric Cancer. *N Engl J Med* 2018; **378**: 1085-1095 [*RCA*] [PMID: 29562147 DOI: 10.1056/NEJMoa1708423] [FullText]

96 **Al-Fakhrany OM**, Elekhnawy E. Helicobacter pylori in the post-antibiotics era: from virulence factors to new drug targets and therapeutic agents. *Arch Microbiol* 2023; **205**: 301 [*RCA*] [PMID: 37550555 DOI: 10.1007/s00203-023-03639-0] [FullText]

97 **Yunle K**, Tong W, Jiyang L, Guojun W. Advances in Helicobacter pylori vaccine research: From candidate antigens to adjuvants-A review. *Helicobacter* 2024; **29**: e13034 [*RCA*] [PMID: 37971157 DOI: 10.1111/hel.13034] [FullText]

98 **Frost I**, Sati H, Garcia-Vello P, Hasso-Agopsowicz M, Lienhardt C, Gigante V, Beyer P. The role of bacterial vaccines in the fight against antimicrobial resistance: an analysis of the preclinical and clinical development pipeline. *Lancet Microbe* 2023; **4**: e113-e125 [*RCA*] [PMID: 36528040 DOI: 10.1016/S2666-5247(22)00303-2] [FullText] [Full Text(PDF)]

99 **Dos Santos Viana I**, Cordeiro Santos ML, Santos Marques H, Lima de Souza Gonçalves V, Bittencourt de Brito B, França da Silva FA, Oliveira E Silva N, Dantas Pinheiro F, Fernandes Teixeira A, Tanajura Costa D, Oliveira Souza B, Lima Souza C, Vasconcelos Oliveira M, Freire de Melo F. Vaccine development against Helicobacter pylori: from ideal antigens to the current landscape. *Expert Rev Vaccines* 2021; **20**: 989-999 [*RCA*] [PMID: 34139141 DOI: 10.1080/14760584.2021.1945450] [FullText]

100 **Patrad E**, Khalighfard S, Amiriani T, Khori V, Alizadeh AM. Molecular mechanisms underlying the action of carcinogens in gastric cancer with a glimpse into targeted therapy. *Cell Oncol (Dordr)* 2022; **45**: 1073-1117 [*RCA*] [PMID: 36149600 DOI: 10.1007/s13402-022-00715-3] [FullText]

101 **Sexton RE**, Al Hallak MN, Diab M, Azmi AS. Gastric cancer: a comprehensive review of current and future treatment strategies. *Cancer Metastasis Rev* 2020; **39**: 1179-1203 [*RCA*] [PMID: 32894370 DOI: 10.1007/s10555-020-09925-3] [FullText]

102 **Vahidi S**, Mirzajani E, Norollahi SE, Aziminezhad M, Samadani AA. Performance of DNA Methylation on the Molecular Pathogenesis of Helicobacter pylori in Gastric Cancer; targeted therapy approach. *J Pharmacopuncture* 2022; **25**: 88-100 [*RCA*] [PMID: 35837145 DOI: 10.3831/KPI.2022.25.2.88] [FullText] [Full Text(PDF)]

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REVIEW

Primary biliary cholangitis: A historical perspective from xanthomatous lesions to modern molecular biology

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Abstract

Primary biliary cholangitis (PBC) is a chronic cholestatic liver disease characterized by damage and loss of the epithelial lining of small intrahepatic bile ducts, leading to ductopenia and cholestasis. In advanced stages, this process results in cirrhosis and liver failure. The disease belongs to cholangiopathies. The review addressed historical questions concerning: The history of the first mention of this disease; how its nomenclature was formed; when specific serological tests were discovered and their importance in the diagnosis of PBC; the history of ursodeoxycholic and other bile acids for the treatment of PBC; and the significance of modern data on impaired bicarbonate production by cholangiocytes in the pathogenesis of PBC.

Key Words: Nonobstructive biliary cirrhosis; Addison-Gull syndrome; La cirrhose hypertrophique avec ictère chronique; Hypertrophic cirrhosis Hanot; Xanthomatous biliary cirrhosis; Primary biliary cirrhosis; Primary biliary cholangitis

Core Tip: The present review was devoted to an examination of the historical aspects of primary biliary cholangitis. The initial references to the disease are examined, along with the evolution of its nomenclature. The importance of the development and the evolution of clinical, laboratory, and instrumental methods for the discovery and elucidation of the pathophysiological mechanisms of primary biliary cholangitis is shown.

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INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic cholestatic progressive liver disease characterized by the destruction, necrosis, and apoptosis of the biliary epithelium predominantly in the intralobular, interlobular, and septal bile ducts with the formation of antimitochondrial autoantibodies (an autoimmune component). In the terminal stage, liver cirrhosis develops.

The advent of new scientific methodologies has been instrumental in the identification of this disease, the initial characterization of its clinical, pathologo-anatomical, morphological, and laboratory manifestations, and the establishment of diagnostic techniques. From these positions we propose the consideration of the evolution of the historical view underlying the discovery, the description of the salient features of this disease, and the establishment of its diagnostic criteria.

EARLY DESCRIPTIONS OF BILIARY CIRRHOSIS

Significance of clinical and pathologo-anatomical methodologies in the identification of disease

An early description of one of the cutaneous signs of the disease now known as PBC dates back to the mid-nineteenth century. In 1851, an article "On a Certain Affectation of the Skin-Vitiligoidea-alpha plana, beta tuberosa" published by Thomas Addison and William Gull in the Guys Hospital Report was the first description of cutaneous clinical signs that the authors observed to be associated with liver pathology[1].

Thomas Addison (1793-1860) was an eminent English scientist, clinician, pathophysiologist, and anatomist. Thomas Addison, after graduating from the Faculty of Medicine at Edinburgh University in 1815, moved to London and took up residency at the Venereal Hospital. He subsequently continued his training at Guy's Hospital, ultimately becoming a lecturer in practical medicine. Thomas Addison was considered an excellent diagnostician but was also a reserved person. He suffered from a depressive disorder and died by suicide on June 29, 1860[2,3].

Sir William Withey Gull, 1st Baronet (1816-1890) was a physician of considerable erudition and is regarded as one of the foremost medical experts of the 19th century. He was the personal physician to Queen Victoria. Sir William Gull was a highly regarded internist and a physician who devoted a great deal of time to his patients. In 1871, he successfully cured the Prince of Wales, who had fallen seriously ill with typhoid fever complicated by bronchitis, and was subsequently appointed as one of Queen Victoria's physicians. As well as Addison, he worked and lectured to medical students at Guy's Hospital and later became the hospital's manager. Gull's scientific interests were in the study of skin diseases[3].

The authors drew attention to some unusual, rare skin lesions that differed from the then-known vitiligo and keloid. The authors introduced the term "Vitiligoidea" to describe them, presenting two forms of these changes: The former, designated "alpha plana", manifested as flat plaque-like formations around the eyes (referred to as xanthelasmas in contemporary nomenclature); and the latter, termed "beta tuberosa", presented as flat tuberous tubercles at sites of trauma and other regions of the body, including the ears, elbows, knees, and skin folds (now categorized as xanthomas) [3].

Xanthelasma has been previously documented. In 1826, Rayer published an atlas of skin diseases in which he first described xanthelasms around the eyes as yellow plaques the color of chamois leather, slightly raised, neither warm nor red, and sometimes disposed in a somewhat symmetrical manner[4]. However, until Addison and Gull no one had associated their occurrence with liver disease.

Addison and Gull described the morphology of xanthomas and xanthelasms and illustrated them with artistic reproductions. One such illustration is presented in the review of White and MacDonald[2], where the artist carefully painted flat formations rising above the skin (xanthelasms) on the upper and lower eyelids of a 43-year-old patient who "...during the last five years there has been a gradual change in the integument of the eyelids, giving her a strange expression..."[1,2].

A total of five observations were recorded and described; three of which bore a resemblance to the disease known today as PBC. The observed patients ranged in age from 24 to 43 years. Subsequent analysis of the descriptions of these observations showed that the skin changes detected in one female corresponded to tuberous sclerosis and in another to an eruptive xanthoma in diabetes mellitus[2]. In three females the presence of flat xanthomas and xanthelasmas on the skin was accompanied by prolonged jaundice, skin itching, and discomfort in the right subcostal area[2].

Despite the unavailability of lifetime histological examination to ascertain the involvement of the liver in the pathological process during that era, Addison and Gull rightly hypothesized the correlation between the appearance of "alpha plana" and "beta tuberosa" with liver damage in these patients[1,5]. Addison and Gull proposed the nomenclature "nonobstructive biliary cirrhosis" for the disorder of liver function manifested by skin changes in the form of "alpha plana" and "beta tuberosa". In light of the seminal contributions made by Addison and Gull in establishing the correlation between cutaneous manifestations and hepatic impairment, the disease was delineated not solely as "nonobstructive biliary cirrhosis" but also as "Addison-Gull syndrome"[6].

In the ensuing years, a series of clinical and experimental papers have been published in the English, French, and German scientific literature, thereby confirming this relationship[4,7-9]. It is noteworthy that some of these works assume a primary role of xanthomatosis in the occurrence of biliary obstruction and the development of biliary cirrhosis[7,10,11]. Subsequent studies of autopsy material did not reveal xanthomatous changes in the epithelial lining of the bile ducts, which was written about by Maxon, Face, and Pure-Smith[7,10,11].

Victor Hanot and the emergence of the term hypertrophic cirrhosis with chronic jaundice (la cirrhose hypertrophique avec ictère chronique)

It is widely accepted that the initial description of the clinical manifestation of the disease that is now recognized as PBC was provided by Victor Charles Hanot, a French physician who was renowned for his contributions to the field of hepatology[4].

Victor Charles Hanot (1844-1896) was one of the most prominent French physicians of the 19th century. Hanot made significant contributions to research in the field of hepatic cirrhosis and hemochromatosis. He was awarded his doctorate from the University of Paris in 1875, following the presentation of his thesis on the subject of "Hypertrophic cirrhosis with chronic jaundice" ("La cirrhose hypertrophique avec ictère chronique")[4,12,13].

In his thesis, Hanot emphasized that the disease described by Addison and Gull was characterized by significant liver enlargement and prolonged jaundice. Hanot pointed out that in contrast to the primary liver enlargement observed in cases of portal cirrhosis the liver enlargement in the present context was not primary. However, it is permanent and persists throughout the course of the disease, without regression afterwards. The analysis of pathologo-anatomical data revealed that the liver enlargement and prolonged jaundice are accompanied by extensive fibrosis[9,12,13]. Subsequent analysis revealed fibrosis to be a hallmark of the disease[14].

Hanot noted that despite the absence of any visible gross obstruction of hepatic ducts, the main cause of the developing chronic process in the liver was the obstruction of bile outflow at the level of small intrahepatic bile ducts, which was confirmed by anatomical and morphological correspondence during autopsy of patients after death[4,12,13]. Hanot hypothesized that the obstruction of bile flow was most likely due to catarrhal inflammation of the smallest branches of the biliary system, resulting in intrahepatic rather than extrahepatic obstruction[4,12,13]. As demonstrated by the observations of Hanot and the works of other authors, it is evident that as the disease progresses, there is cellular infiltration and fibroblastic proliferation around the small bile ducts. Hanot's merit lies in the fact that he clearly delineated the clinical picture of the disease, which is different from that of secondary biliary cirrhosis[13]. Concurrently, erroneous attributions were made concerning the potential role of syphilis in its etiology[12,13]. In addition to the term "hypertrophic cirrhosis with chronic jaundice", the terms "hypertrophic cirrhosis Hanot" and "Hanot's disease" were also utilized[5].

IMPORTANCE OF DEVELOPING CHEMICAL AND LABORATORY METHODS FOR STUDY OF THE DISEASE

Determination of the composition of xanthomas, xanthelasms, changes in blood lipid composition, and the emergence of the term "xanthomatous biliary cirrhosis"

The first report pertaining to the chemical composition of xanthomas and xanthelasms was made by Quinquaud in Paris in 1878 during a meeting of the clinical society[15]. As stated in Quinquaud's published report, the occurrence of xanthomas and xanthelasms has been observed "...in cases of excess fat in the blood...". According to the author's data, the content of "fats" in the blood of such patients can exceed the physiological norm by six or more times[15]. On the basis of the data obtained, the author concluded that "...changes in blood composition play an important role in the development of this curious condition".

In subsequent years, an escalating number of scientific studies described clinical observations of hepatic impairment accompanied by protracted jaundice and concomitant cutaneous manifestations in the form of xanthomas and xanthelasms[7,16-20]. By the end of the 19th century, the characterization of the disease was based on clinical manifestations and the results of pathological autopsies of deceased patients[4]. In the first half of the 20th century, new laboratory methods were developed and subsequently integrated into clinical practice, thus enabling the identification and recognition of laboratory signs of disease in patients diagnosed with Addison-Gull syndrome/Hanot's disease. The assessment of lipid metabolism and determination of a lipid profile has enabled the development of a more comprehensive understanding of the mechanisms underpinning the formation of xanthomas and xanthelasma.

Thannhauser and his colleagues[20-23] in the late 1930s and early 1940s revealed a clear relationship between the appearance of xanthomas and xanthelasma with elevated levels of cholesterol and lecithin (four to eight times higher than normal) in the serum of these patients based on clinical signs and taking into account biochemical data. The authors suggested that the fundamental cause of the aforementioned condition is an imbalance in the processes of cholesterol formation and excretion[23]. Despite a substantial increase in cholesterol and lecithin levels in the blood serum, it remained clear and did not appear creamy[20,24]. Important laboratory signs of Addison-Gull Syndrome/Hanot's Disease included: Elevated plasma cholesterol and lecithin levels in these patients.

In addition to describing new laboratory signs, the authors made significant additions to the known (presence of xanthomas and xanthelasma on the skin, enlargement of the liver and spleen, jaundice of multi-year duration) clinical signs[21-23]. Thannhauser and his colleagues[20] noted that: (1) "...all cases reported so far..." were represented by female patients between 30 and 50 years of age[25-30]; (2) Skin itching was one of the most important symptoms of the disease in

these patients; and (3) despite Hanot's suggestion of small bile duct inflammation, the patients did not have the fever and/or chills characteristic of inflammation.

Taking into account the presence of xanthomas and xanthelasms in patients with cirrhosis associated with Addison-Gull Syndrome/Hanot's Disease, MacMahon and Thannhauser[20] in 1949 proposed to the name "xanthomatous biliary cirrhosis" for this disease, distinguishing it as an independent pathology. In this study, the authors noted that "xanthomatous biliary cirrhosis" could not be classified in the "hypercholesterolemic familial xanthomatosis" group because "...no familial morbidity has been identified in the cases that have been previously described"[20].

The subject of generalized lipid accumulation and the development of xanthomatosis, first mentioned by Quinquaud[4,15], was developed further by Thannhauser and his colleagues[21-23]. These works attracted the attention of the renowned 20th-century lipidologist Ahrens[31].

Ahrens and his proposed term "primary biliary cirrhosis"

The contributions of Ahrens[32] to the field of cholesterol metabolism have been significant, particularly in the area of understanding the impairment of lipid metabolism in liver disease, which is accompanied by xanthomatosis and hyperlipidemia.

Ahrens (1915-2000) was a famous lipidologist of the 20th century. Ahrens received his Bachelor of Science (1937) and Doctor of Medicine (1941) degrees from Harvard University[33]. *Throughout the course of his professional career, Ahrens was a known proponent of patient-centered research. He is best known for his book "The Crisis of Clinical Research" published in 1992. It examined the transition of medical science to laboratory research. In his work, he demonstrated the significance of comprehending the physicochemical characteristics of diverse classes of lipoproteins for various diseases. The clinical study that he conducted on the subject of lipid profiles in patients suffering from primary biliary cirrhosis is the most frequently cited by other researchers within the relevant field*[24].

The findings of his research constituted the foundation for the advancement of diagnostic methodologies pertaining to "xanthomatous biliary cirrhosis", which ensured his diagnosis at earlier stages.

Ahrens' observations of 18 patients with "xanthomatous biliary cirrhosis" revealed that while there was an increase in serum lipids, this was not accompanied by the presence of xanthomas and xanthelasms. This finding suggested the existence of a pre-xanthomatous stage in these patients despite the development of cholestasis. The pre-xanthomatous stage, as defined by Ahrens, is characterized by a lesser degree of manifestation of biliary tract obstruction and a less pronounced increase in serum lipid levels[24]. Ahrens' research demonstrated that the majority of patients are in the pre-xanthomatous stage and that the xanthomatous phase, as he defined it, signified only the most severe and dramatic terminal stage of the disease's progression. According to Ahrens xanthomatosis in this disease was secondary, and the disease progresses through three stages: Pre-xanthomatous; xanthomatous; and terminal stages.

Ahrens also demonstrated the possibility of reverse development of xanthomas and xanthelasms. The progression to a marked xanthomatous stage and back depends on the degree of increase in serum cholesterol and lecithin levels. It has been shown that if serum lipids remain sufficiently elevated for a long period of time, the appearance of skin xanthomas can be predicted. If for any reason the obstruction is removed, serum lipids can be expected to fall to normal levels and the xanthomas and xanthelasms to dissipate[24].

At the same time, according to Ahrens, the changes in the lipid profile leading to skin xanthomatosis are strikingly different from those that define the presence of arterial atheromatosis. Therefore, no clinical, electrocardiographic, or radiographic signs of atherosclerosis or coronary artery disease were detected in these patients[24,34].

Ahrens[20,24] expressed concerns about the imprecision of the term "xanthomatous biliary cirrhosis" proposed by Thannhauser and colleagues due to the fact that xanthomas and xanthelasms occur in only 5%-10% of cases. A large proportion of early reports described patients with "xanthomatous biliary cirrhosis" at the terminal stage of disease. As a rule, the disease was diagnosed at late stages of pronounced cholestasis[31,33,35]. As a result, Ahrens *et al*[31], taking into account the morphological description of the final cirrhotic stage of the disease in 1950, justified and introduced the term "primary biliary cirrhosis" (PBC). Subsequent to that period, the terms "Addison-Gull syndrome" and "cirrhosis of Hanot" have become historical.

Ahrens emphasized the necessity of identifying specific criteria for the differential diagnosis of PBC (in which there is damage to small intralobular, interlobular, and intrahepatic bile ducts) and secondary biliary cirrhosis (initiated by obstructive lesions)[31]. The author drew attention to the paradox in blood lipid analysis in primary and secondary biliary cirrhosis, which he proposed to use for differential diagnosis of these conditions.

A comprehensive analysis of the literature data, encompassing long-term clinical and laboratory observations of patients diagnosed with PBC and "secondary biliary cirrhosis", has shown: (1) In PBC the large bile ducts are always passable and bile can flow into the intestine through them, but extremely high values of total cholesterol (4-8 times higher than the reference value) as well as lecithin (4-10 times higher than the reference value) in serum are noted[20,24]. At the same time, neutral lipids are at low values; and (2) In "secondary biliary cirrhosis" associated with complete obstruction of large bile ducts and impaired bile outflow, there is no such significant increase in cholesterol and lecithin level [20,24].

According to Ahrens, serum lipid analysis, in addition to clinical findings, is the decisive factor for the diagnosis of "primary (xanthomatous) biliary cirrhosis". The clinical and pathological features of PBC have been supplemented with laboratory tests. In the 1950s this approach promoted some successes in improving the diagnosis of this disease at earlier stages. The revealed disorders of lipid status in PBC were confirmed by the data on the study of bile and hepatobioptates of these patients using ^{31}P-NMR spectroscopy[36]. By the close of the 1950s, the prerequisites for studies of the histopathological evolution of the disease had been established[3,37,38].

Along with the emergence of new laboratory methods of investigation, morphological methods were being developed and improved. The incorporation of percutaneous puncture liver biopsy into clinical practice has engendered the capacity to undertake lifetime histochemical and morphological studies of liver biopsy specimens. This, in turn, has facilitated the

enhancement of diagnostic capabilities, enabling the identification of disease at its earliest stages.

MORPHOLOGICAL AND ELECTRON MICROSCOPIC METHODS IN THE STUDY OF PBC

Morphological classifications of PBC

In the 1960s Rubin *et al*[39] first described the spectrum of histological changes of the liver detected in "primary (xanthomatous) biliary cirrhosis" in liver biopsy specimens. The authors noted that the primary damage occurs in the smallest bile ducts, which is accompanied by a cellular, granulomatous reaction in the portal tract and in the connective tissue where there are lymphatic vessels[39-42]. Bile thrombi are detected in intralobular bile ducts, *i.e.* in cholangioles and canaliculi. The surfaces of hepatocytes in close proximity to damaged canaliculi undergo degenerative and even necrotic changes[39,41]. For the majority of the duration of the disease, the patient appears to be in good health[4]. This is due to the fact that the liver cells are only slightly damaged, and the effects of portal hypertension and hepatic failure are only evident in the later stages of the disease. It is only at this point, after many years, that the disease ultimately results in death.

The advent of lifetime morphological and histochemical studies of liver biopsy specimens has led to the establishment of morphological classifications that delineate the developmental stages of PBC. In 1967 Scheuer[37] presented a detailed description of the four histological stages of this disease (Table 1)[38]. A little later Popper and Schaffner[43] and Schaffner and Bacchin[44] presented a comprehensive analysis of the morphological changes that occur at various stages of the disease, with particular emphasis on their correlation with the clinical manifestations of cholestasis. The authors underscored the significance of these morphological changes in relation to the disturbances in bile acid metabolism[44]. In 1978 Ludwig *et al*[38] having analyzed 219 individual biopsy specimens from 101 patients with the established morphological diagnosis of "chronic non-purulent destructive cholangitis" (the syndrome of PBC) proposed their classification of the stages of the disease (Table 1).

The use of different terminology in the nomenclature of the individual morphological stages of PBC serves to emphasize, reflect, and clarify the essence of the ongoing pathological processes without contradicting each other. The classification system has been in continuous use for many decades; this is partly due to the fact that the descriptions of the stages in these classifications are based on uniform histological criteria that apply to all specimens and are easily reproducible. All classifications concur that in the early stages the principal changes occur in the small bile ducts. It is only as cholestasis progresses that hepatocyte damage occurs and fibrosis develops (stage three).

The famous pathomorphologist Professor Aruin has noted that the morphological changes in liver biopsy specimens are characterized by a peculiar 'mosaic' of damage[45]. Signs of at least two different stages can always be seen in histological preparations. This phenomenon can be attributed to the fact that the sequential progression from one stage to another may be subject to differential expression in different areas of the liver[45,46].

Electron microscopic changes in PBC

Electron microscopic and electron histochemical studies of liver biopsy material from patients diagnosed with PBC revealed stereotypical changes in ultrastructure that are characteristic of the unfolded cholestasis stage: Local divergence of hepatocytes with an increase in intercellular spaces; partial hypertrophy and vacuolization of smooth and rough endoplasmic reticulum of hepatocytes; accumulation of lipid droplets in liver cells and formation of myelin-like structures; and changes in the shape and structure of mitochondria, characterized by a decrease in the number of inner membrane cristae[46]. The osmiophilic, myelin-like, and cholesterol-digitonin structures are likely to represent intracellular complexes of cholesterol, phospholipids, and bile acids formed in response to cholestasis[46]. It can be hypothesized that osmiophilic, myelin-like, and cholesterol-digitonin structures represent micellar-lamellar complexes of bile acids with cholesterol and phospholipids. Indirectly, the presence of these structures indicates that the damage of hepatocytes in chronic cholestasis is caused by the accumulation of bile acids in them.

The data from electron microscopic studies demonstrating the disturbance of intercellular contacts in cholestasis syndrome are in agreement with the results of cell adhesion assessment. A quantitative adhesiometric method was developed for the study of hepatobioptates in patients with various chronic liver diseases[47]. The application of this method has revealed that in PBC there is a weakening of interhepatocyte interactions (contacts) due to increased pressure in bile capillaries resulting from intrahepatic cholestasis in small bile ducts[47].

As illustrated in Table 1, the allocation of four stages of the disease is accepted by all morphologists, but only the fourth stage of the disease is universally presented by all as cirrhosis and corresponds to the generally accepted criteria of this disease[45].

Sheila Sherlock, analysis and critique of the term PBC

Given that cirrhosis in these patients develops only in the late stages of the disease, the world-renowned scientist, hepatologist Sheila Sherlock, already in 1959 began to oppose the term "primary biliary cirrhosis" proposed by Ahrens.

Sherlock[35] *(1918-2001) was a world-renowned physician, scientist, gastroenterologist, and a leading hepatologist. In 1945, Sherlock*[35] *was awarded the Edinburgh MD degree (and gold medal) for her thesis on "The Liver in Disease: With special reference to aspiration biopsy of the liver". At the age of 30, she established the world's first liver disease unit. In 1950, she cofounded the American Association for the Study of the Liver with Hans Popper, and in 1958, she became the first president of the International Association for the Study of the Liver. Sherlock*[35] *published an outstanding monograph in 1955, entitled "Diseases of the Liver and Biliary System". This monograph has been reprinted many times (eight editions during her lifetime). It has also been translated into at least six languages, demonstrating its international appeal and importance*[48,49].

Table 1 The morphological classification of stages of primary biliary cholangitis, adapted from Ludwig *et al*[38]

Ref.	Morphologic findings on biopsy			
	Stage I	Stage II	Stage III	Stage IV
Scheuer[37]	*Portal* Portal hepatitis	*Periportal* Periportal hepatitis	*Septal* Bridging necrosis (passive septa) or septal fibrosis (active septa), or both	*Cirrhosis*
Popper and Schaffner[43]	*Cholangitis* Portal hepatitis with duct lesions	*Ductular proliferation* Same as stage II of Scheuer	*Precirrhosis* Same as stage III of Scheuer	*Cirrhosis*
Ludwig *et al* [38]	*Florid duct lesion* Portal hepatitis with duct lesions, or periportal hepatitis with duct lesions but without ductular proliferation	*Ductular proliferation* Periportal hepatitis (and bridging necrosis?), with ductular proliferation but without fibrosis	*Scarring* Septal fibrosis but absence of true regenerative nodules	*Cirrhosis*

In the course of long-term observation of patients diagnosed with PBC, Sherlock[35] showed that in most cases the disease proceeds without the presence of cirrhosis, thus allowing her to distinguish the pre-cirrhotic stage of the disease [50]. She described 42 clinical observations of patients diagnosed with PBC who she supervised for 15 years[35]. In describing the clinical features, Sherlock carefully described the timing of appearance of skin itching, jaundice, the sites of xanthomas and xanthelasma depending on the level of plasma cholesterol elevation, as well as laboratory abnormalities of alkaline phosphatase, gamma glutamyl transpeptidase (GGT) activity, and histological changes ascertained from examination of liver biopsy and/or autopsy material.

According to her descriptions, in a significant portion of patients (20 patients, 48%) skin itching appeared long before the appearance of jaundice (from several months to 11 years), but in 14 patients (33%) itching appeared against the background of pronounced jaundice. Xanthomas on the extensor surfaces of elbows, wrists, buttocks, knees, and ankles were observed in 20 patients (48%), all with high serum cholesterol. Six cases of patients had flat xanthelasma on the eyelids. In several cases hepatomegaly was diagnosed in the absence of any specific complaints (this may have been the first description of an asymptomatic course of the disease). In no case was fever or abdominal pain syndrome observed. The diagnosis was usually verified after surgical intervention (37 patients, 88%!!!) or by liver biopsy. The latter was performed in few patients, as it was still a very rare procedure in the early 1950s, but one that Sherlock successfully mastered.

An examination of biopsy and autopsy material revealed that the majority of patients did not exhibit the conventional pattern of cirrhosis and disruption of the liver lobule architecture[35]. It was emphasized that in the early stages of PBC, nodular regeneration is inconspicuous. Fibrosis development is only noted in the later stages, at which point it becomes evident that the zonal architecture is distorted, creating a "…mixed picture of portal and biliary cirrhosis". The absence for many years of clinical and morphological signs of cirrhosis led Sherlock to propose the term "chronic intrahepatic obstructive jaundice" for this disease[35]. However, the term proposed by Sherlock[35] was not widely supported and accepted. Meanwhile, Ahrens' term, PBC, continued to be used until 2015.

During the mid-1960s, clinical immunology and immunological techniques underwent rapid development, which proved to be a significant moment for the diagnosis of the earliest and most asymptomatic stages of the disease.

IMMUNOLOGICAL METHODS IN THE STUDY OF PBC

Development of immunology and its significance in the study and diagnosis of PBC

By the mid-1960s, most of the clinical, biochemical, and histopathological features of PBC had been established. Concurrently, the field of immunology was undergoing rapid development with the discovery of immunoglobulin classes and subtypes and the introduction of new immunological methods. During the same time, the hypothesis that PBC may have an immunological basis was put forward[3].

Using immunological methods Paronetto *et al*[51] found and described antibodies directed against protein components of the bile ducts. These antibodies were found in cases of viral hepatitis (67%), secondary biliary cirrhosis (43%), extrahepatic biliary obstruction (32%), and alcoholic cirrhosis (22%)[52].

Concurrently, Deborah Doniach, a prominent immunologist in Britain, approached the equally distinguished Sherlock with a proposal to conduct immunological testing on patients diagnosed with PBC.

Deborah Doniach (1912-2004) was a world-renowned scientist, a British clinical immunologist, and a pioneer in the field of autoimmune diseases. She finished the Royal Free Medical School and started working in an organized department of immunology. Doniach was a pioneer in the establishment of new paradigms in immunology. She had extremely fruitful collaborations with many talented scientists and clinicians, including Sherlock and Geoffrey Walker. This collaboration has been extremely fruitful and has identified the presence of antimitochondrial antibodies (AMA) in patients with PBC as one of the pathognomonic features. Doniach soon became an Honorary Consultant Immunopathologist and Professor of Clinical Immunology.

Doniach's proposal was motivated by the observation that some patients with this disease manifest Hashimoto's thyroiditis, characterized by the presence of circulating anti-thyroglobulin antibodies[3]. In experimental models of thyroiditis, rabbits have been observed to exhibit lymphocytic infiltration, which is analogous to that observed in liver

biopsy specimens from patients with PBC[53]. Sherlock agreed and delegated the execution of immunological studies in PBC to Geoffrey Walker, a trainee under her supervision who demonstrated a keen interest in the emerging field of immunology[3].

The study used an immunofluorescence test with sera from 32 patients with PBC and showed non-specific cytoplasmic fluorescence in unfixed sections of thyroid, stomach, kidney, and peripheral blood lymphocytes[52]. Concurrently, the sera of 33 patients in the control group who had biliary obstruction or suffered from other types of cholestasis exhibited negative results in the immunofluorescence test[52]. The study revealed that the antibodies detected in the serum of patients with PBC exhibited neither species nor organ specificity.

Subsequent studies demonstrated that the autoantibodies detected were directed against subcellular organelles or soluble cellular proteins, which were present not only in the liver but also in numerous other organs[54]. Later immunological studies demonstrated that the antigen that reacted with sera from patients diagnosed with PBC was predominantly located within the mitochondrial fraction of tissue homogenates obtained by differential centrifugation[55]. Furthermore, the research conducted by Berg *et al*[55] demonstrated that the antigen was situated on the inner membrane of mitochondria. The findings obtained by Walker, Doniach, and Sherlock constituted a seminal discovery[52,55,56]. This study identified the presence of AMAs in patients with PBC as one of the pathognomonic features.

The subsequent 25 years of research concentrated on the identification of the antigen to which mitochondrial antibodies are formed. It had been hypothesized that the antigen was associated with mitochondrial ATPase[57]. However, subsequent research revealed that pure ATPase fractions prepared by alternative methods exhibited no reactivity with the sera of patients diagnosed with PBC[58,59]. The employment of immunological methodologies has enabled the identification of nine distinct types of antibodies, each directed towards varying antigenic structures of mitochondria (M1-M9)[60]. It has been identified that M2 antigenic components are specific for the sera of patients with PBC[61]. In addition to M2, other mitochondrial antigens and antibodies to them have been shown to have diagnostic and prognostic significance for PBC[62]. Detection of anti-M9 is associated with the early stages of the disease[63]. Mitochondrial anti-M4 and anti-M8 are detected exclusively in M2 positive patients[62]. Their appearance in the serum of patients with PBC is associated with a marked progression of the disease[64].

The development and application of molecular genetic techniques has allowed the identification of M2 antigen as the E2 subunit of the pyruvate dehydrogenase (PDH) complex[63]. The antigen that reacts with the serum of patients with PBC has been characterized as a lipoprotein localized on the inner mitochondrial membrane[65]. This was later confirmed by electron microscopic studies by Bianchi *et al*[66]. The specificity of antibodies to this antigen for the diagnosis of PBC was striking[58].

The significance of the discovery of AMAs for the diagnosis of PBC

Walker *et al*[52] demonstrated that the diagnostic value of other tests in differentiating between PBC and biliary obstruction was extremely limited[56]. The presence of AMA was detectable by indirect immunofluorescence and has been observed in most of the patients diagnosed with PBC[67]. It was therefore recommended that mitochondrial antibody tests be used as an alternative to surgery for the purpose of confirming a diagnosis of PBC[50,68].

In addition to AMA antinuclear antibodies were detected in a proportion of patients with PBC[69]. The presence of AMA and antinuclear antibodies was the reason to consider PBC as a prototype of autoimmune disease and to describe it as a paradigmatic model of autoimmune disease[70] that some authors are currently questioning.

The presence of a significant AMA titer (> 1:40) has been demonstrated to provide strong evidence for the diagnosis of PBC, even in the absence of symptoms and with normal serum alkaline phosphatase levels[71]. Typical, early histological signs of PBC can be detected by examining liver biopsy specimens from individuals whose sole manifestation of the disease is a positive serum AMA test[71]. The true prevalence of asymptomatic and subclinical cases of the disease became evident following the determination of AMAs and the wider availability of routine screening of liver biochemical tests[72].

The identification of features pertaining to the manifestation of initial signs and the progression of the disease formed the foundation for the establishment of clinical classifications of stages and variants in the course of PBC.

Classification of clinical stages and variants in the course of PBC

In 1984, Proka, a graduate student of academician Loginov, distinguished the following variants of the initial manifestations of PBC in his PhD thesis, based on the initial manifestations of the disease[73]: (1) The disease commences with symptoms of cholestasis but without jaundice (the most prevalent form of the onset of PBC); (2) The onset of the disease was characterized by nonspecific symptoms and manifestations (cholecystic and dyspeptic, hematological, articular-muscular, and other manifestations); and (3) The disease begins with a combination of skin itching and jaundice (the picture of advanced cholestasis).

The classification proposed by Sasaki *et al*[74] in 1985 most completely, concisely, and accurately reflected the stages of the clinical course of the disease in the majority of patients suffering from PBC: (1) Asymptomatic stage; (2) Pruritus stage; (3) Jaundice stage; and (4) Terminal stage.

A more detailed description of the stages of the course of PBC was presented in the classification of Poupon[75], published in 1991: (1) Preclinical, asymptomatic stage (characterized by the appearance of AMA in the blood, moderate increases in GGT, alkaline phosphatase, and 5'-nucleotidase activity); (2) Clinical stage of the disease (usually lasting from 5 to 19 years and characterized by the manifestation of all clinical signs of PBC); and (3) Terminal stage of the disease with the development of fibrosis, cirrhosis, and their complications (portal hypertension, ascites, esophageal-gastric bleeding).

Treatment

The enhanced diagnostic capacity for PBC, coupled with the presumed autoimmune nature of the condition, has given rise to the use of corticosteroids[76] and immunosuppressants[77-79]. According to a number of authors, the use of corticosteroids was led to a decrease in weakness, intensity of skin itching, a slight decrease of plasma alkaline phosphatase activity[76,80], and even accompanied by some positive alteration in the histological picture of the liver[77]. However, as experience with corticosteroids in treating patients with PBC was accumulated, it became apparent that there was no unequivocal positive therapeutic effect as seen in other autoimmune diseases. According to Mann[4] since he first initiated the use of corticosteroids to treat patients with PBC in 1960, he has had the impression that their use may have a favorable effect on the course of the disease. However, he still tends to consider that corticosteroids are of limited or negligible therapeutic effect.

The first encouraging results of immunosuppressants[77] such as azathioprine[81], cyclosporine[82], and other drugs including colchicine[83,84], methotrexate[85,86], and chlorambucil[87] were not confirmed in randomized controlled trials. The lack of a therapeutic effect and the emergence of serious side effects have led to the abandonment of further use of these medications for the treatment of PBC.

Copper metabolism disorder and associated cutaneous hyperpigmentation in PBC[88,89] prompted the use of D-penicillamine in these patients[90]. However, the use of D-penicillamine in randomized controlled trials has not confirmed positive results with its use[90]. This is due to the fact that copper in PBC accumulates in the organs and tissues of the body in a non-toxic, ceruloplasmin-bound form, and the use of the drug is associated with a number of adverse side effects[89,90].

Cutaneous pruritus is one of the early clinical signs of PBC that requires treatment but is often difficult to manage. The use of opioid receptor antagonists (*e.g.*, naloxone, naltrexone, *etc.*)[91], ion-exchange resins (*e.g.*, cholestyramine, cholestipol, *etc.*), phenobarbital, and rifampicin[92,93] helps to reduce the intensity of skin itching observed in this disease. Rifampicin has been recommended as an alternative second-line drug for the treatment of cutaneous pruritus associated with chronic cholestasis. But given its hepatotoxicity, it should not be used long-term and in patients with stages 3 and 4 PBC. The data available in the literature on the use of these drugs suggest that they are all useful for short-term relief of pruritus, although the mechanism of this effect remains unknown[93]. Recently, it has been thought that endogenous opioids may modulate signaling pathways involved in hepatic pruritus; however, they are unlikely to be major pruritogenic factors in liver disease[94].

Ursodeoxycholic acid as a first-line drug in the treatment of PBC

In 1987 the German hepatologists Leuschner and Kurtz[95] reported a positive effect of ursodeoxycholic acid (UDCA) in patients with PBC. The drug has been studied in many randomized, placebo-controlled trials in patients with stages I-IV of PBC, with both positive and equivocal results[96-101]. Clinical studies have shown that oral administration of UDCA at a dose of 13-15 mg/kg/day is well tolerated by patients and has a positive therapeutic effect in cholestatic liver diseases[102]. Scientific publications have noted that UDCA improves biochemical markers of cholestasis (alkaline phosphatase, GGT) in most patients, slows disease progression, delays liver transplantation and death in most patients, and improves survival rates[103,104].

Subsequent studies have demonstrated a direct correlation between the efficacy of UDCA use and the stage of the disease, with the therapeutic response being more pronounced in cases where treatment is initiated at an earlier stage (stage I-II). The administration of UDCA in PBC has been shown to slow down the progression of histological manifestations and prolong patient survival without the necessity for liver transplantation. Consequently, this medication is currently recommended as a first-line therapeutic option for all patients with PBC[102,105,106]. The precise mechanism through which UDCA renders a positive effect remains to be fully elucidated. Nevertheless, it is evident that this effect is dependent on its physicochemical properties, in addition to its metabolic processes and enterohepatic circulation[107,108].

For 35 years UDCA has been a unique agent of choice for the treatment of patients with PBC. In recent years, there has been an expansion in the list of hydrophilic bile acids that are used to treat cholestatic liver disease, including PBC. In addition to UDCA, the potential use of obeticholic acid, tauroursodeoxycholic acid, and norursodeoxycholic acid as pharmaceutical agents has also been considered[107,108].

Advancements in the domain of liver transplantation techniques have been instrumental in extending the lifespan of patients afflicted with terminal PBC[109,110]. Despite the emergence of drug therapy, liver transplantation continues to be a prominent treatment option for patients with advanced disease. Liver transplantation has been demonstrated to be a highly efficacious therapeutic modality for individuals afflicted with end-stage PBC[109,110].

NOMENCLATURE CHANGE FROM CIRRHOSIS TO CHOLANGITIS

The presence of asymptomatic preclinical stage, in conjunction with the protracted absence of any physical indications of the disease, served to substantiate the inaccuracy and ineligibility of the nomenclature PBC, a conclusion that had previously been emphasized by Sherlock[35,111]. Therefore, the 2nd monothematic conference of the European Association for the Study of the Liver dedicated to PBC was held on May 23-24, 2014 in Milan, Italy. At the conference, it was proposed to change the nomenclature from "primary biliary cirrhosis" to "primary biliary cholangitis". Two important arguments were put forward in favor of this proposal.

The term "primary biliary cholangitis" is more accurate in reflecting the processes occurring during the development of the disease, which includes damage to biliary epithelial cells of small intrahepatic bile ducts, with the subsequent development of ductulopenia, intrahepatic cholestasis and slowly progressive fibrosis. The disease is characterized by elevated alkaline phosphatase activity, the presence of AMAs, and the manifestation of a specific histological picture in a liver biopsy. In the asymptomatic and clinical stages, according to morphological and clinical criteria, patients do not have signs of cirrhosis. The development of cirrhosis is considered to be a late terminal stage of the disease, and therefore the term primary biliary cholangitis is more correct.

The term PBC is also more acceptable to patients. The word "cirrhosis" is perceived by the majority of patients as a fatal, terminal illness with death sentence. From a psychological standpoint, the diagnosis of primary biliary cirrhosis instills a sense of dread in patients, as it is often perceived to portend a poor prognosis, which can exacerbate its course and the weighting of the condition, particularly in its early stages. At present, the course of the disease usually has a slowly progressive course, accompanied by a gradual deterioration in patient quality of life. At the same time, treatment requires lifelong medication. However, with scientific progress in recognizing new mechanisms of the disease and the efforts of scientists to develop new drugs, there is hope that patients can live with the disease rather than die from it.

Following a period of deliberation, the conference participants indicated their support for the proposal of the Board of the European Association for the Study of the Liver to effect a change in the nomenclature from primary biliary cirrhosis to primary biliary cholangitis. The decision under discussion was supported and endorsed by a number of professional associations and organizations during the period 2014-2015. These included the American Association for the Study of Liver Disease, the American Gastroenterological Association, the United European Gastroenterology Governing Board and the Asian Pacific Association for the Study of the Liver. The decision of all public organizations was submitted to the World Health Organization, which also expressed support for the decision to change the nomenclature of primary biliary cirrhosis to primary biliary cholangitis without changing the abbreviation of the disease. The World Health Organization further recommended adjustments to the relevant code of the International Classification of Diseases 10th (K.74.3) and 11th (DB37.2) revisions. Since 2015 the disease has been recognized by the global medical community as primary biliary cholangitis.

ADVENT OF NOVEL DATA AND THE EVOLUTION OF CONTEMPORARY REPRESENTATIONS PERTAINING TO PBC

Despite the fact that the etiology of PBC has not yet been identified, a significant volume of scientific data has been accumulated to date, allowing the formation of hypotheses regarding the mechanisms that trigger the processes of damage to small biliary epithelial cells (cholangiocytes), the formation of AMA, and the development of the first clinical signs of the disease.

Because PBC affects predominantly females, a genetic study has been conducted that is dedicated to the contribution of the X chromosome to the genetics of PBC. In 2021 the first report on this topic was published[112]. A number of genes have been identified that the authors express as possibly contributing, each with modest effects, to the development of this disease[112].

Data obtained using molecular genetic techniques have demonstrated that X-linked epigenetic alterations are induced in females with PBC for unknown reasons, leading to increased expression of microRNA 506 in cholangiocytes[113]. The latter regulates the functioning of transmembrane proteins responsible for the flow of bicarbonate from the cholangiocyte into the lumen of the bile duct. The suppression of the expression of type 3 receptor to inositol triphosphate and chlorine/bicarbonate anion exchanger 2 (AE2) was observed in liver biopsy specimens and blood mononuclear cells of patients with PBC. The resulting suppression of type 3 receptor to inositol triphosphate and AE2 activity leads to insufficient bicarbonate entry into the bile duct lumen[113-115]. An insufficient supply of bicarbonate into bile ducts in PBC results in a shift of the pH of intraductal (hepatic) bile to the slightly acidic region and an increase of pH inside cholangiocytes to the slightly alkaline region.

The discovery and analysis of these data allowed the development of the concept of the pathogenesis of damage to small biliary epithelial cells, with subsequent development of the initial clinical and laboratory signs of PBC[89,116,117]. The presented concept was a pioneering advancement in the field, as it facilitated the understanding of the underlying mechanisms that underpin the emergence of the initial indications of PBC in the asymptomatic stage of the disease. In addition, it allows a response to the following research questions: (1) Why are only cholangiocytes that line small and medium-sized bile ducts susceptible to damage in PBC?[115,118-120]; (2) How does the E2 antigen of the PDH complex, located on the inner mitochondrial membrane, undergo immunomodification and become accessible to immune cells, thus facilitating the subsequent antibody formation?[117]; (3) Why are antibodies selective formed only to the E2 subunit, while the E1 and E3 subunits of PDH immunotolerance is remain?[117]; and (4) What is the mechanism of AMA formation?[117].

This concept provides a clear indication that not AMA but the accumulation of bile acids within small cholangiocytes in the PBC is the primary damaging factor. Accumulation of bile acids in small biliary epithelial cells and their incomplete apoptosis redirected to necrosis can lead to damage of membrane structures and their death, with subsequent development of ductulopenia and cholestasis[116,121]. As cholestasis intensifies, hepatocytes are involved in the pathological process, which is accompanied by the gradual development of fibrosis and cirrhosis.

The treatment of PBC remains challenging as the cause of this chronic, slowly progressive cholestatic liver disease has not been identified. The advent of novel scientific data has the potential to inform future new drug development, with the objective of local suppression of microRNA 506 activity or activation of the AE2 anion exchanger in cholangiocytes. This

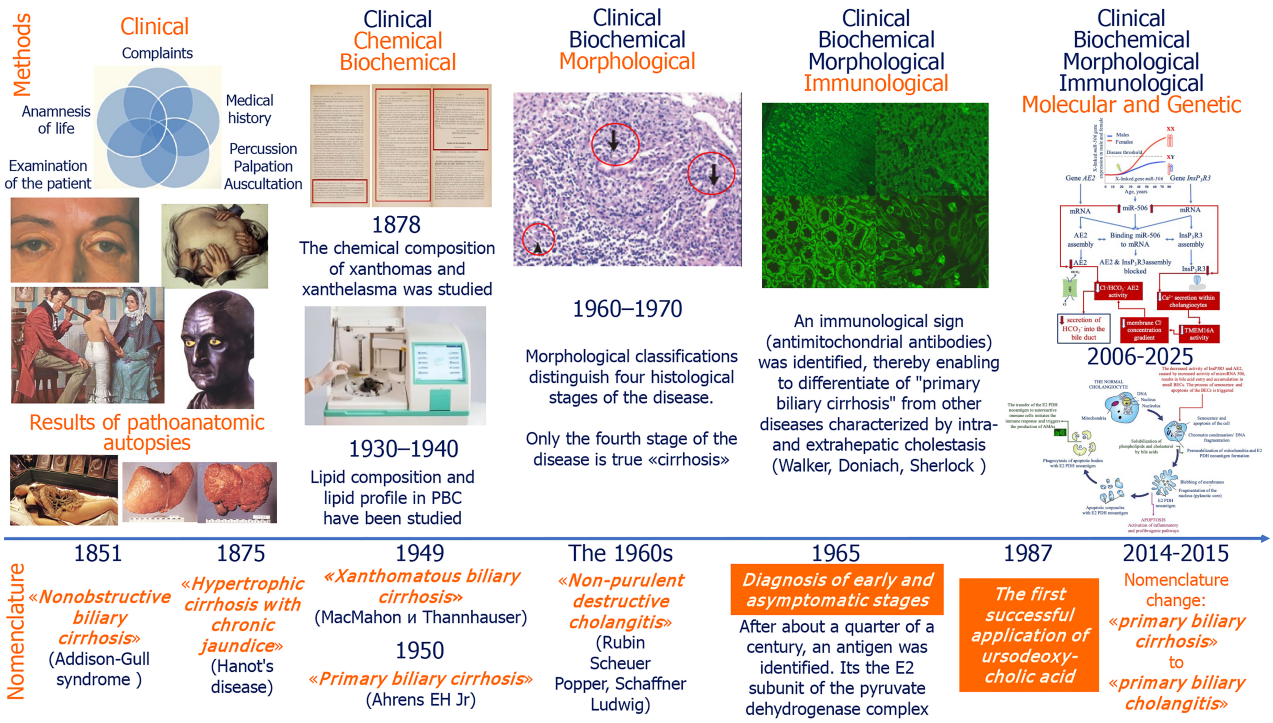

Figure 1 The significance of methods in the discovery and study of primary biliary cholangitis.

is likely to be one of the new therapeutic trends in the treatment of PBC in addition to or to replace the existing bile acid drugs.

CONCLUSION

PBC is a chronic cholestatic liver disease belonging to cholangiopathies. Almost 175 years ago, the initial descriptions of the individual signs of the disease were made using solely clinical methods, such as interviews and examinations, and the results of pathological anatomical autopsies (Figure 1).

The advent of novel scientific research methods has facilitated a more profound comprehension of the progression of the disease and the subsequent body changes, thereby paving the way for the potential enhancement of diagnostic methods. The historical stages of disclosure and description of various signs, mechanisms of their development, outlined in this review, demonstrate the importance and contribution of laboratory (chemical, biochemical, morphological, immunological, molecular-genetic, and others) and instrumental (percutaneous puncture liver biopsy) methods in the study of PBC. The advent of immunology and the development of immunological research methodologies have had a significant impact on the diagnosis of this disease. The identification of antimitochondrial autoantibodies, in conjunction with elevated activity of alkaline phosphatase and GGT, facilitates the diagnosis of PBC in the asymptomatic stage of the disease.

In spite of the fact that the etiology of this disease is not known, disclosure of pathogenetic mechanisms involved in PBC progression promoted development of modern ways of treatment of this disease, including use of bile acid preparations (UDCA, tauroursodeoxycholic acid, obeticholic acid, norusodeoxycholic acid). In the near future, research efforts directed towards elucidating the etiology and pathogenesis of the disease are expected to result in the development of novel pharmacotherapeutic agents capable of halting its progression and preventing its occurrence.

FOOTNOTES

Author contributions: Reshetnyak VI developed the conceptualization and design of the study, the selection and analysis of scientific literature, and the authorship, critical checking, and editing of the review; Vinnitskaya EV contributed to the selection and analysis of scientific literature and the writing of the review; Maev IV contributed to the conceptualization and design of the study and the drafting, critical review, and editing of the review; All authors provided approval for the final version of the review.

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REFERENCES

1 **Addison T**, Gull W. On a certain affection of the skin vitiligoidea a plana, b tuberosa. *Guy's Hosp Rep* 1851; **7**: 265-276
2 **White IR**, MacDonald DM. Thomas Addison, M.D. First dermatologist at Guy's Hospital. *J Am Acad Dermatol* 1982; **6**: 426-430 [*RCA*] [PMID: 7040509 DOI: 10.1016/s0190-9622(82)80302-2] [FullText]
3 **Reuben A**. The serology of the Addison-Gull syndrome. *Hepatology* 2003; **37**: 225-228 [*RCA*] [PMID: 12500211 DOI: 10.1002/hep.510370134] [FullText]
4 **Mann WN**. Biliary cirrhosis: an appraisal. The Croonian Lecture 1975. *J R Coll Physicians Lond* 1976; **10**: 117-132 [PMID: 1107528 DOI: 10.1016/S0035-8819(25)03052-1] [FullText]
5 **Osadchuk AM**, Garanina AA, Kurtov IV, Betaneli TSh. Liver diseases that have eponymic names: from the past to the present. *Astrakhan Med J* 2018; **13**: 62-73 [DOI: 10.17021/2018.13.4.62.73] [FullText]
6 **Heathcote EJ**. Primary biliary cirrhosis: historical perspective. *Clin Liver Dis* 2003; **7**: 735-740 [*RCA*] [PMID: 14594128 DOI: 10.1016/s1089-3261(03)00098-9] [FullText]
7 **Moxon W**. Jaundice and xanthelasma. Trans. Path. Soc. London. 1873; 24: 129
8 **Legg JW**. The Liver in Jaundice. *Br Med J* 1874; **1**: 607-608 [*RCA*] [PMID: 20747496 DOI: 10.1136/bmj.1.697.607] [FullText]
9 **Legg JW**. The Liver in Jaundice. *Br Med J* 1874; **1**: 645-646 [*RCA*] [PMID: 20747504 DOI: 10.1136/bmj.1.698.645] [FullText]
10 **Fagge CH**. General xanthelasma or vitiligoidea, Trans. Path. Soc. London. 1872-1873; 24: 242
11 **Pye-Smith PH**. Xanthelasma of skin, peritoneum and mucous membrane associated with jaundice, Trans. Path. Soc. London. 1873; 24: 250
12 **Hanot VC**. Etude sur une forme de Cirrhose Hypertrophique du Foie (cirrhose hypertrophique avec ictère chronique). Paris. 851-859. Available from: https://archive.org/details/b22296621
13 **Hanot V**. La cirrhose hypertrophique avec ictère chronique. Paris, France: Rueff et Cie Editeurs; 1892. Available from: https://archive.org/details/lacirrhosehypert00hano/page/18/mode/2up
14 **Wilensky AO**. The cause and pathogenetic mechanism of hypertrophic cirrhosis of the liver of the Hanot type. *Am J Dig Dis* 1947; **14**: 74-77 [*RCA*] [PMID: 20286590 DOI: 10.1007/BF03001487] [FullText]
15 **Quinquaud M**. Bulletin de la Societe Clinique de Paris. 1878; 2: 259. Available from: https://archive.org/details/s2939id1396789/page/260/mode/2up
16 **Balzer F**. Sur l'étoile de xanthoma. Arch, de physiol. norm, et path. 1884; 4: 6. Available from: https://archive.org/details/sim_jama-dermatology_1921-10_4_4
17 **Hardaway W**. Disappearance of extensive eruption of xanthoma. *Dis* 1890; **8**: 21
18 **Futcher TB**. XANTHELASMA AND CHRONIC JAUNDICE. *Am J Med Sci* 1905; **130**: 939-950 [*RCA*] [DOI: 10.1097/00000441-190512000-00001] [FullText]
19 **Chvostek F**. Xanthelasma und Icterus. Ztschr. f. klin. *Med* 1911; **73**: 76
20 **Macmahon HE**, Thannhauser SJ. Xanthomatous biliary cirrhosis; a clinical syndrome. *Ann Intern Med* 1949; **30**: 121-179 [*RCA*] [PMID: 18106239 DOI: 10.7326/0003-4819-30-1-121] [FullText]
21 **Thannhauser SJ**, Magendantz H. An article contributed to an anniversary volume in honor of doctor joseph hersey pratt: The different clinical groups of xanthomatous diseases: A clinical physiological study of 22 cases. *Ann Intern Med* 1938; **11**: 1662-1746 [DOI: 10.7326/0003-4819-11-9-1662] [FullText]
22 **Thannhauser SJ**. Lipidoses: Diseases of the Cellular Lipid Metabolism. Oxford University Press, 1940; 88 Available from: https://www.amazon.com/Lipidoses-Diseases-Cellular-Lipid-Metabolism/dp/B000NX40F0
23 **Thannhauser SJ**, Schmidt G. Lipins and lipidoses. *Physiol Rev* 1946; **26**: 275-317 [*RCA*] [PMID: 20982971 DOI: 10.1152/physrev.1946.26.2.275] [FullText]
24 **Ahrens EH Jr**, Kunkel HG. The relationship between serum lipids and skin xanthomata in 18 patients with primary biliary cirrhosis. *J Clin Invest* 1949; **28**: 1565-1574 [*RCA*] [PMID: 15395959 DOI: 10.1172/JCI102222] [FullText]
25 **Comfort MW**, Shepard VHM and Snell AM. Xanthomatous biliary cirrhosis. *Proc. Staff Meet Mayo Clin* 1941; **16**: 374-377
26 **Herbert FK**. A case of juvenile xanthomatosis: With Enlarged Liver and Spleen and Greatly Increased Plasma Lipoids. *Arch Dis Child* 1943; **18**: 41-49 [*RCA*] [PMID: 21032245 DOI: 10.1136/adc.18.93.41] [FullText]
27 **Eusterman GB**, Montgomery H. Disorders of the liver and the extrahepatic biliary ducts associated with cutaneous xanthoma and hyperlipemia. *Gastroenterology* 1944; **3**: 275
28 **Layani F**, Laudat M, Astruc P. Sur un cas de maladie xanthomateuse. Bull. et mem. Soc. med. d. hop. de Paris. 1939; 4: 355
29 **Hoffbauer F**, Evans G, Watson C. Cirrhosis of the Liver Presenting the Clinical Features of Xantho-matous Biliary Cirrhosis, But with Confirmation at Autopsy (Follow-up of Case Reported Previously*). *Med Clin North Am* 1945; **29**: 1054-1055 [DOI: 10.1016/s0025-7125(16)36075-8] [FullText]
30 **Gephardt MC**. Xanthomatous biliary cirrhosis; lipid levels while receiving inositol. *Ann Intern Med* 1947; **26**: 764-769 [*RCA*] [PMID:

20238917 DOI: 10.7326/0003-4819-26-5-764] [FullText]
31 **Ahrens EH Jr**, Payne MA, Kunkel HG, Eisenmenger WJ, Blondheim SH. Primary biliary cirrhosis. *Medicine (Baltimore)* 1950; **29**: 299-364 [*RCA*] [PMID: 14796348 DOI: 10.1097/00005792-195012000-00002] [FullText]
32 **Ahrens EH Jr**. Special issue dedicated in memory of Dr. Edward H. Ahrens, Jr. *Cardiovasc Drug Rev* 2002; **20**: 237-343 [*RCA*] [PMID: 12481196] [FullText]
33 **Hirsch J**. A tribute to Pete Ahrens. *J Lipid Res* 2001; **42**: 891-893 [*RCA*] [PMID: 11369795 DOI: 10.1016/S0022-2275(20)31611-4] [FullText]
34 **Reshetnyak VI**, Maev IV. Features of Lipid Metabolism Disorders in Primary Biliary Cholangitis. *Biomedicines* 2022; **10** [*RCA*] [PMID: 36551803 DOI: 10.3390/biomedicines10123046] [FullText]
35 **Sherlock S**. Primary billiary cirrhosis (chronic intrahepatic obstructive jaundice). *Gastroenterology* 1959; **37**: 574-586 [PMID: 14445875 DOI: 10.1016/S0016-5085(19)35377-6] [FullText]
36 **Khristianovich DS**, Reshetniak VI, Loginov AS, Iushmanov VE, Tumanian MA. [31P-NMR spectroscopy of the human liver and bile]. *Biull Eksp Biol Med* 1988; **106**: 678-681 [*RCA*] [PMID: 3207874 DOI: 10.1007/BF00802173] [FullText]
37 **Scheuer P**. Primary biliary cirrhosis. *Proc R Soc Med* 1967; **60**: 1257-1260 [*RCA*] [PMID: 6066569 DOI: 10.1177/003591576706001205] [FullText] [Full Text(PDF)]
38 **Ludwig J**, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch A Pathol Anat Histol* 1978; **379**: 103-112 [*RCA*] [PMID: 150690 DOI: 10.1007/BF00432479] [FullText]
39 **Rubin E**, Schaffner F, Popper H. Primary biliary cirrhosis. Chronic non-suppurative destructive cholangitis. *Am J Pathol* 1965; **46**: 387-407 [*RCA*] [PMID: 14266218] [FullText]
40 **Popper H**, Schaffner F, Barka T. Has proliferation of bile ductules clinical significance? *Acta Hepatosplenol* 1962; **9**: 129-139 [*RCA*] [PMID: 14487989] [FullText]
41 **Popper H**, Rubin E, Schaffner F. The problem of primary biliary cirrhosis. *Am J Med* 1962; **33**: 807-810 [*RCA*] [PMID: 13972306 DOI: 10.1016/0002-9343(62)90213-9] [FullText]
42 **Popper H**, Schaffner F. Fine structural changes of the liver. *Ann Intern Med* 1963; **59**: 674-691 [*RCA*] [PMID: 14082719 DOI: 10.7326/0003-4819-59-5-674] [FullText]
43 **Popper H**, Schaffner F. Pathophysiology of cholestasis. *Hum Pathol* 1970; **1**: 1-24 [*RCA*] [PMID: 4940286 DOI: 10.1016/s0046-8177(70)80002-8] [FullText]
44 **Schaffner F**, Bacchin PG, Hutterer F, Scharnbeck HH, Sarkozi LL, Denk H, Popper H. Mechanism of cholestasis. 4. Structural and biochemical changes in the liver and serum in rats after bile duct ligation. *Gastroenterology* 1971; **60**: 888-897 [PMID: 5581332 DOI: 10.1016/S0016-5085(71)80089-6] [FullText]
45 **Loginov AS**, Aruin LI. Clinical morphology of the liver. Moscow: Meditsina Publishers; 1985; 240 Available from: https://www.libex.ru/dimg/4894a.jpg
46 **Reshetnyak VI**. Concept on the pathogenesis and treatment of primary biliary cirrhosis. *World J Gastroenterol* 2006; **12**: 7250-7262 [*RCA*] [PMID: 17143938 DOI: 10.3748/wjg.v12.i45.7250] [FullText] [Full Text(PDF)]
47 **Loginov AS**, Iamskova VP, Tumanova NB, Tkachev VD, Reshetnak VI. [Study of hepatocyte adhesion in chronic diffuse diseases of the liver]. *Biull Eksp Biol Med* 1989; **108**: 160-162 [*RCA*] [PMID: 2679907 DOI: 10.1007/BF00840644] [FullText]
48 **McIntyre N**. Sheila: Professor Dame Sheila Sherlock (1918-2001). *Hepatology* 2002; **35**: 507-509 [*RCA*] [PMID: 11870360 DOI: 10.1053/jhep.2002.0350507] [FullText]
49 **Guardia J**, Esteban R. [In memorian of Sheila Sherlock]. *Med Clin (Barc)* 2002; **118**: 250 [*RCA*] [PMID: 11882276 DOI: 10.1016/s0025-7753(02)72351-8] [FullText]
50 **Sherlock S**. Diseases of the liver and biliary system. Blackwell Scientific Publications, 1975: 874 Available from: https://archive.org/details/diseasesofliverb00sher/page/n5/mode/2up
51 **Paronetto F**, Schaffner F, Mutter RD, Kniffen JC, Popper H. Circulating antibodies to bile ductular cells in various liver diseases. *JAMA* 1964; **187**: 503-506 [*RCA*] [PMID: 14084823 DOI: 10.1001/jama.1964.03060200035006] [FullText]
52 **Walker JG**, Doniach D, Roitt IM, Sherlock S. Serological tests in diagnosis of primary biliary cirrhosis. *Lancet* 1965; **1**: 827-831 [*RCA*] [PMID: 14263538 DOI: 10.1016/s0140-6736(65)91372-3] [FullText]
53 **Rose NR**, Witebsky E. Studies on organ specificity. V. Changes in the thyroid glands of rabbits following active immunization with rabbit thyroid extracts. *J Immunol* 1956; **76**: 417-427 [*RCA*] [PMID: 13332243 DOI: 10.4049/jimmunol.76.6.417] [FullText]
54 **Walker G**. The immunology of liver disorders. *Proc R Soc Med* 1974; **67**: 566-573 [*RCA*] [PMID: 4605272 DOI: 10.1177/003591577406P210] [FullText] [Full Text(PDF)]
55 **Berg PA**, Doniach D, Roitt IM. Mitochondrial antibodies in primary biliary cirrhosis. I. Localization of the antigen to mitochondrial membranes. *J Exp Med* 1967; **126**: 277-290 [*RCA*] [PMID: 4165742 DOI: 10.1084/jem.126.2.277] [FullText] [Full Text(PDF)]
56 **Doniach D**, Roitt IM, Walker JG, Sherlock S. Tissue antibodies in primary biliary cirrhosis, active chronic (lupoid) hepatitis, cryptogenic cirrhosis and other liver diseases and their clinical implications. *Clin Exp Immunol* 1966; **1**: 237-262 [*RCA*] [PMID: 5330183] [FullText]
57 **Sayers T**, Leoutsakos A, Berg P, Baum H. Antimitochondrial antibodies (AMA) in primary biliary cirrhosis. I. Separation of the PBC antigen activity from mitochondrial ATPase activity. *J Bioenerg Biomembr* 1981; **13**: 255-267 [*RCA*] [PMID: 6460755 DOI: 10.1007/BF00743204] [FullText]
58 **Baum H**, Berg PA. The complex nature of mitochondrial antibodies and their relation to primary biliary cirrhosis. *Semin Liver Dis* 1981; **1**: 309-321 [*RCA*] [PMID: 6180480 DOI: 10.1055/s-2008-1040734] [FullText]
59 **Leoutsakos A**, Baum H, Sanadi DR. Evidence that mitochondrial coupling factor B might be the major antigen in primary biliary cirrhosis. Abstract XI International Congress of Biochemistry. 1979; 526
60 **Meek F**, Khoury EL, Doniach D, Baum H. Mitochondrial antibodies in chronic liver diseases and connective tissue disorders: further characterization of the autoantigens. *Clin Exp Immunol* 1980; **41**: 43-54 [*RCA*] [PMID: 6449333] [FullText]
61 **Berg PA**, Klein R, Lindenborn-Fotinos J. Antimitochondrial antibodies in primary biliary cirrhosis. *J Hepatol* 1986; **2**: 123-131 [*RCA*] [PMID: 2419388 DOI: 10.1016/s0168-8278(86)80015-0] [FullText]
62 **Loginov AS**, Tsaregorodtseva TM, Zotina MM. The immune system and digestive diseases. Moscow: Meditsina Publishers. 1986: 42-65 Available from: https://archive.org/details/1986_20221114
63 **Bassendine MF**, Fussey SP, Mutimer DJ, James OF, Yeaman SJ. Identification and characterization of four M2 mitochondrial autoantigens in primary biliary cirrhosis. *Semin Liver Dis* 1989; **9**: 124-131 [*RCA*] [PMID: 2471276 DOI: 10.1055/s-2008-1040503] [FullText]

64 **Weber P**, Brenner J, Stechemesser E, Klein R, Weckenmann U, Klöppel G, Kirchhof M, Fintelmann V, Berg PA. Characterization and clinical relevance of a new complement-fixing antibody--anti-M8--in patients with primary biliary cirrhosis. *Hepatology* 1986; **6**: 553-559 [*RCA*] [PMID: 3089894 DOI: 10.1002/hep.1840060402] [FullText]

65 **Berg PA**, Roitt IM, Doniach D, Cooper HM. Mitochondrial antibodies in primary biliary cirrhosis. IV. Significance of membrane structure for the complement-fixing antigen. *Immunology* 1969; **17**: 281-293 [*RCA*] [PMID: 5804537] [FullText]

66 **Bianchi FB**, Penfold PL, Roitt IM. Mitochondrial antibodies in primary biliary cirrhosis. V. Ultrastructural localization of the antigen to the inner mitochondrial membrane using a direct peroxidase conjugate. *Br J Exp Pathol* 1973; **54**: 652-657 [*RCA*] [PMID: 4131502] [FullText]

67 **Munoz LE**, Thomas HC, Scheuer PJ, Doniach D, Sherlock S. Is mitochondrial antibody diagnostic of primary biliary cirrhosis? *Gut* 1981; **22**: 136-140 [*RCA*] [PMID: 7215944 DOI: 10.1136/gut.22.2.136] [FullText]

68 **Klatskin G**, Kantor FS. Mitochondrial antibody in primary biliary cirrhosis and other diseases. *Ann Intern Med* 1972; **77**: 533-541 [*RCA*] [PMID: 4566006 DOI: 10.7326/0003-4819-77-4-533] [FullText]

69 **Berg PA**, Klein R. Autoantibodies in primary biliary cirrhosis. *Springer Semin Immunopathol* 1990; **12**: 85-99 [*RCA*] [PMID: 2195682 DOI: 10.1007/BF00192685] [FullText]

70 **Gershwin ME**, Mackay IR. Primary biliary cirrhosis: paradigm or paradox for autoimmunity. *Gastroenterology* 1991; **100**: 822-833 [*RCA*] [PMID: 1993507 DOI: 10.1016/0016-5085(91)80033-6] [FullText]

71 **Mitchison HC**, Bassendine MF, Hendrick A, Bennett MK, Bird G, Watson AJ, James OF. Positive antimitochondrial antibody but normal alkaline phosphatase: is this primary biliary cirrhosis? *Hepatology* 1986; **6**: 1279-1284 [*RCA*] [PMID: 3793004 DOI: 10.1002/hep.1840060609] [FullText]

72 **James OF**, Bhopal R, Howel D, Gray J, Burt AD, Metcalf JV. Primary biliary cirrhosis once rare, now common in the United Kingdom? *Hepatology* 1999; **30**: 390-394 [*RCA*] [PMID: 10421645 DOI: 10.1002/hep.510300213] [FullText]

73 **Reshetnyak VI**. Mechanisms of bile formation and primary biliary cirrhosis. Moscow: Publishing house "Red square", 2003: 144 Available from: http://irbis.svlib.ru/cgi-bin/irbis64r_14/cgiirbis_64.exe?LNG=&Z21ID=&I21DBN=IBIS&P21DBN=IBIS&S21STN=1&S21REF=&S21FMT=fullwebr&C21COM=S&S21CNR=10&S21P01=0&S21P02=1&S21P03=A=&S21STR=Решетняк%2C%20Василий%20Иванович

74 **Sasaki H**, Inoue K, Higuchi K, Yasuyama T, Koyata H, Kuroki T, Yamamoto S, Ichida F. Primary biliary cirrhosis in Japan: national survey by the Subcommittee on Autoimmune hepatitis. *Gastroenterol Jpn* 1985; **20**: 476-485 [*RCA*] [PMID: 4076730 DOI: 10.1007/BF02774786] [FullText]

75 **Poupon R**. [Primary biliary cirrhosis]. *Rev Prat* 1991; **41**: 1161-1165 [*RCA*] [PMID: 2063116] [FullText]

76 **Mitchison HC**, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. *J Hepatol* 1992; **15**: 336-344 [*RCA*] [PMID: 1447500 DOI: 10.1016/0168-8278(92)90065-w] [FullText]

77 **Sherlock S**. Immunosuppressive therapy in chronic liver disease. *Minerva Med* 1978; **69**: 2605-2609 [*RCA*] [PMID: 714260] [FullText]

78 **Beukers R**, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. *J Hepatol* 1992; **14**: 1-6 [*RCA*] [PMID: 1737909 DOI: 10.1016/0168-8278(92)90123-7] [FullText]

79 **Bray GP**, Williams R. Immunointervention in primary biliary cirrhosis: an overview. *J Autoimmun* 1992; **5** Suppl A: 293-300 [*RCA*] [PMID: 1503625 DOI: 10.1016/0896-8411(92)90047-t] [FullText]

80 **Mutimer DJ**, Bassendine MF, Crook P, James OF. Vasculitis in primary biliary cirrhosis--response to prednisolone. *Q J Med* 1990; **75**: 509-513 [PMID: 2388998] [FullText]

81 **Christensen E**, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B, Doniach D, Ranek L, Tygstrup N, Williams R. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. *Gastroenterology* 1985; **89**: 1084-1091 [*RCA*] [PMID: 3899841 DOI: 10.1016/0016-5085(85)90213-6] [FullText]

82 **Wiesner RH**, Ludwig J, Lindor KD, Jorgensen RA, Baldus WP, Homburger HA, Dickson ER. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. *N Engl J Med* 1990; **322**: 1419-1424 [*RCA*] [PMID: 2184355 DOI: 10.1056/NEJM199005173222003] [FullText]

83 **Kaplan MM**, Alling DW, Zimmerman HJ, Wolfe HJ, Sepersky RA, Hirsch GS, Elta GH, Glick KA, Eagen KA. A prospective trial of colchicine for primary biliary cirrhosis. *N Engl J Med* 1986; **315**: 1448-1454 [*RCA*] [PMID: 3537784 DOI: 10.1056/NEJM198612043152304] [FullText]

84 **Warnes TW**. Colchicine in primary biliary cirrhosis. *Aliment Pharmacol Ther* 1991; **5**: 321-329 [*RCA*] [PMID: 1777543 DOI: 10.1111/j.1365-2036.1991.tb00035.x] [FullText]

85 **Kaplan MM**, Knox TA. Treatment of primary biliary cirrhosis with low-dose weekly methotrexate. *Gastroenterology* 1991; **101**: 1332-1338 [*RCA*] [PMID: 1936805 DOI: 10.1016/0016-5085(91)90085-y] [FullText]

86 **Kaplan MM**, Knox TA. Methotrexate for primary biliary cirrhosis. *Gastroenterology* 1992; **102**: 1824 [*RCA*] [PMID: 1568598 DOI: 10.1016/0016-5085(92)91761-r] [FullText]

87 **Hoofnagle JH**, Davis GL, Schafer DF, Peters M, Avigan MI, Pappas SC, Hanson RG, Minuk GY, Dusheiko GM, Campbell G. Randomized trial of chlorambucil for primary biliary cirrhosis. *Gastroenterology* 1986; **91**: 1327-1334 [*RCA*] [PMID: 3533699 DOI: 10.1016/0016-5085(86)90183-6] [FullText]

88 **Smallwood RA**, Williams HA, Rosenoer VM, Sherlock S. Liver-copper levels in liver disease: studies using neutron activation analysis. *Lancet* 1968; **2**: 1310-1313 [*RCA*] [PMID: 4177386 DOI: 10.1016/s0140-6736(68)91814-x] [FullText]

89 **Reshetnyak VI**, Maev IV. Pathophysiology of biochemical signs of primary biliary cholangitis. *Explor Dig Dis* 2023 [DOI: 10.37349/edd.2023.00024] [FullText]

90 **Stavinoha MW**, Soloway RD. Current therapy of chronic liver disease. *Drugs* 1990; **39**: 814-840 [*RCA*] [PMID: 2196164 DOI: 10.2165/00003495-199039060-00002] [FullText]

91 **Thornton JR**, Losowsky MS. Opioid peptides and primary biliary cirrhosis. *BMJ* 1988; **297**: 1501-1504 [*RCA*] [PMID: 3147046 DOI: 10.1136/bmj.297.6662.1501] [FullText]

92 **Ghent CN**, Carruthers SG. Treatment of pruritus in primary biliary cirrhosis with rifampin. Results of a double-blind, crossover, randomized trial. *Gastroenterology* 1988; **94**: 488-493 [*RCA*] [PMID: 3275568 DOI: 10.1016/0016-5085(88)90442-8] [FullText]

93 **Loginov AS**, Reshetniak VI, Petrakov AV. [The treatment of primary biliary liver cirrhosis with rifampicin]. *Ter Arkh* 1993; **65**: 57-62 [*RCA*] [PMID: 8211805] [FullText]

94 **Düll MM**, Wolf K, Vetter M, Dietrich P, Neurath MF, Kremer AE. Endogenous Opioid Levels Do Not Correlate With Itch Intensity and Therapeutic Interventions in Hepatic Pruritus. *Front Med (Lausanne)* 2021; **8**: 641163 [*RCA*] [PMID: 33937284 DOI:

10.3389/fmed.2021.641163] [FullText] [Full Text(PDF)]

95 **Leuschner U**, Kurtz W. Treatment of primary biliary cirrhosis and cholestatic disorders with ursodeoxycholic acid. *Lancet* 1987; **2**: 508 [*RCA*] [PMID: 2887794 DOI: 10.1016/s0140-6736(87)91812-5] [FullText]

96 **Lindor KD**, Dickson ER, Baldus WP, Jorgensen RA, Ludwig J, Murtaugh PA, Harrison JM, Wiesner RH, Anderson ML, Lange SM. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. *Gastroenterology* 1994; **106**: 1284-1290 [*RCA*] [PMID: 8174890 DOI: 10.1016/0016-5085(94)90021-3] [FullText]

97 **Heathcote EJ**, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN, Michieletti P, Minuk GY, Pappas SC, Scully LJ. The Canadian Multicenter Double-blind Randomized Controlled Trial of ursodeoxycholic acid in primary biliary cirrhosis. *Hepatology* 1994; **19**: 1149-1156 [*RCA*] [PMID: 8175136 DOI: 10.1002/hep.1840190512] [FullText]

98 **Poupon RE**, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. *N Engl J Med* 1994; **330**: 1342-1347 [*RCA*] [PMID: 8152446 DOI: 10.1056/NEJM199405123301903] [FullText]

99 **Combes B**, Carithers RL Jr, Maddrey WC, Lin D, McDonald MF, Wheeler DE, Eigenbrodt EH, Muñoz SJ, Rubin R, Garcia-Tsao G. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. *Hepatology* 1995; **22**: 759-766 [PMID: 7657280 DOI: 10.1002/hep.1840220311] [FullText]

100 **Loginov AS**, Reshetnyak VI. Treatment of patients with primary biliary cirrhosis with ursodeoxycholic acid. Russian journal of gastroenterology, hepatology, coloproctology. 1994; 4: 64-69

101 **Parés A**, Caballería L, Rodés J, Bruguera M, Rodrigo L, García-Plaza A, Berenguer J, Rodríguez-Martínez D, Mercader J, Velicia R. Long-term effects of ursodeoxycholic acid in primary biliary cirrhosis: results of a double-blind controlled multicentric trial. UDCA-Cooperative Group from the Spanish Association for the Study of the Liver. *J Hepatol* 2000; **32**: 561-566 [*RCA*] [PMID: 10782903 DOI: 10.1016/s0168-8278(00)80216-0] [FullText]

102 **AISF Expert Panel:**. Position paper of the Italian Association for the Study of the Liver (AISF): Management and treatment of primary biliary cholangitis. *Dig Liver Dis* 2024; **56**: 1461-1474 [*RCA*] [PMID: 38902184 DOI: 10.1016/j.dld.2024.05.002] [FullText]

103 **Lindor KD**, Gershwin ME, Poupon R, Kaplan M, Bergasa NV, Heathcote EJ; American Association for Study of Liver Diseases. Primary biliary cirrhosis. *Hepatology* 2009; **50**: 291-308 [*RCA*] [PMID: 19554543 DOI: 10.1002/hep.22906] [FullText]

104 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of cholestatic liver diseases. *J Hepatol* 2009; **51**: 237-267 [*RCA*] [PMID: 19501929 DOI: 10.1016/j.jhep.2009.04.009] [FullText]

105 **Iljinsky IM**, Tsirulnikova OM. Primary biliary cholangitis. *RJTAO* 2021; **23**: 162-170 [DOI: 10.15825/1995-1191-2021-1-162-170] [FullText]

106 **Absandze K**, Vinnitskaya E, Sandler Y, Khaimenova T, Filina D, Saliyev K. Primary Biliary Cholangitis and Incomplete Response to Ursodeoxycholic Acid Therapy: Who is Guilty and What to Do? *EPh* 2022; **18**: 14-18 [DOI: 10.33978/2307-3586-2022-18-22-14-18] [Full Text]

107 **Reshetnyak VI**, Maev IV. Bile acid therapy for primary biliary cholangitis: Pathogenetic validation. *World J Exp Med* 2025; **15**: 101771 [*RCA*] [PMID: 40115760 DOI: 10.5493/wjem.v15.i1.101771] [FullText] [Full Text(PDF)]

108 **Reshetnyak VI**, Maev IV. Bile Acids in Primary Biliary Cholangitis as a Damaging Factor for Cholangiocytes and as Drugs. *Eff Pharmacother* 2024; **20**: 56-66

109 **Aguilar MT**, Carey EJ. Current Status of Liver Transplantation for Primary Biliary Cholangitis. *Clin Liver Dis* 2018; **22**: 613-624 [*RCA*] [PMID: 30259857 DOI: 10.1016/j.cld.2018.03.011] [FullText]

110 **Martin EF**. Liver Transplantation for Primary Biliary Cholangitis. *Clin Liver Dis* 2022; **26**: 765-781 [*RCA*] [PMID: 36270728 DOI: 10.1016/j.cld.2022.06.014] [FullText]

111 **Cheung AC**, Montano-Loza A, Swain M, Vincent C, Renner E, Sherman M, Janssen HL, Mason AL. Time to make the change from 'primary biliary cirrhosis' to 'primary biliary cholangitis'. *Can J Gastroenterol Hepatol* 2015; **29**: 293 [*RCA*] [PMID: 26196152 DOI: 10.1155/2015/764684] [FullText]

112 **Asselta R**, Paraboschi EM, Gerussi A, Cordell HJ, Mells GF, Sandford RN, Jones DE, Nakamura M, Ueno K, Hitomi Y, Kawashima M, Nishida N, Tokunaga K, Nagasaki M, Tanaka A, Tang R, Li Z, Shi Y, Liu X, Xiong M, Hirschfield G, Siminovitch KA; Canadian-US PBC Consortium; Italian PBC Genetics Study Group; UK-PBC Consortium; Japan PBC-GWAS Consortium, Carbone M, Cardamone G, Duga S, Gershwin ME, Seldin MF, Invernizzi P. X Chromosome Contribution to the Genetic Architecture of Primary Biliary Cholangitis. *Gastroenterology* 2021; **160**: 2483-2495.e26 [*RCA*] [PMID: 33675743 DOI: 10.1053/j.gastro.2021.02.061] [FullText]

113 **van Niekerk J**, Kersten R, Beuers U. Role of Bile Acids and the Biliary HCO(3)(-) Umbrella in the Pathogenesis of Primary Biliary Cholangitis. *Clin Liver Dis* 2018; **22**: 457-479 [*RCA*] [PMID: 30259847 DOI: 10.1016/j.cld.2018.03.013] [FullText]

114 **Banales JM**, Prieto J, Medina JF. Cholangiocyte anion exchange and biliary bicarbonate excretion. *World J Gastroenterol* 2006; **12**: 3496-3511 [*RCA*] [PMID: 16773707 DOI: 10.3748/wjg.v12.i22.3496] [FullText] [Full Text(PDF)]

115 **Hohenester S**, Wenniger LM, Paulusma CC, van Vliet SJ, Jefferson DM, Elferink RP, Beuers U. A biliary HCO3- umbrella constitutes a protective mechanism against bile acid-induced injury in human cholangiocytes. *Hepatology* 2012; **55**: 173-183 [*RCA*] [PMID: 21932391 DOI: 10.1002/hep.24691] [FullText]

116 **Reshetnyak VI**, Maev IV. New insights into the pathogenesis of primary biliary cholangitis asymptomatic stage. *World J Gastroenterol* 2023; **29**: 5292-5304 [*RCA*] [PMID: 37899787 DOI: 10.3748/wjg.v29.i37.5292] [FullText] [Full Text(PDF)]

117 **Reshetnyak VI**, Maev IV. Mechanism of formation and significance of antimitochondrial autoantibodies in the pathogenesis of primary biliary cholangitis. *Explor Immunol* 2024 [*RCA*] [DOI: 10.37349/ei.2024.00163] [FullText]

118 **Matsubara T**, Kozaka K, Matsui O, Nakanuma Y, Uesaka K, Inoue D, Yoneda N, Yoshida K, Kitao A, Yokka A, Koda W, Gabata T, Kobayashi S. Peribiliary glands: development, dysfunction, related conditions and imaging findings. *Abdom Radiol (NY)* 2020; **45**: 416-436 [*RCA*] [PMID: 31707436 DOI: 10.1007/s00261-019-02298-4] [FullText]

119 **Maillette de Buy Wenniger LJ**, Hohenester S, Maroni L, van Vliet SJ, Oude Elferink RP, Beuers U. The Cholangiocyte Glycocalyx Stabilizes the 'Biliary HCO3 Umbrella': An Integrated Line of Defense against Toxic Bile Acids. *Dig Dis* 2015; **33**: 397-407 [*RCA*] [PMID: 26045275 DOI: 10.1159/000371864] [FullText]

120 **Prieto J**, Banales JM, Medina JF. Primary biliary cholangitis: pathogenic mechanisms. *Curr Opin Gastroenterol* 2021; **37**: 91-98 [*RCA*] [PMID: 33332913 DOI: 10.1097/MOG.0000000000000703] [FullText] [Full Text(PDF)]

121 **Berg CP**, Stein GM, Keppeler H, Gregor M, Wesselborg S, Lauber K. Apoptosis-associated antigens recognized by autoantibodies in patients with the autoimmune liver disease primary biliary cirrhosis. *Apoptosis* 2008; **13**: 63-75 [*RCA*] [PMID: 18060504 DOI: 10.1007/s10495-007-0157-6] [FullText]

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Pathophysiology of anastomotic stricture following rectal anastomosis: Insights into mechanisms, risk factors, and preventive strategies

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Abstract

Anastomotic stricture (AS) remains a significant complication following rectal anastomosis, with an incidence ranging from 5% to 30% depending on surgical technique, patient factors, and postoperative management. This review aims to elucidate the pathophysiology of AS, exploring the underlying mechanisms that contribute to its development, including ischemia, inflammation, fibrosis, and impaired healing. Key risk factors such as low anterior resection, preoperative radiotherapy, and anastomotic leakage are critically analyzed based on recent clinical and experimental evidence. The article synthesizes current insights into the molecular and cellular processes, such as excessive collagen deposition and myofibroblast activation, that drive stricture formation. Furthermore, preventive strategies, including optimized surgical techniques (*e.g.*, tension-free anastomosis), enhanced perioperative care, and emerging therapeutic interventions (*e.g.*, anti-fibrotic agents), are discussed with an emphasis on translating research into clinical practice. By integrating findings from preclinical studies, clinical trials, and meta-analyses, this review highlights gaps in current knowledge and proposes future directions for research, such as the role of personalized medicine and novel biomaterials in reducing AS incidence. This comprehensive analysis underscores the need for a multidisciplinary approach to mitigate this challenging postoperative complication.

Key Words: Anastomotic stricture; Rectal cancer; Fibrosis; Inflammation; Anastomotic leakage; Radiotherapy; Ischemia; Surgical technique

Core Tip: Anastomotic stricture following rectal anastomosis is a multifactorial complication driven by fibrosis, inflammation, anastomotic leakage, radiotherapy, and ischemia. This review highlights the pathophysiological mechanisms, including excessive collagen deposition and transforming growth factor-beta activation, and identifies key risk factors such as neoadjuvant radiotherapy and surgical technique. Preventive strategies, such as preserving blood supply and using standardized stapling techniques, are emphasized to improve patient outcomes and reduce stricture incidence.

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INTRODUCTION

Anastomotic stricture (AS), or stenosis, is a significant complication following low anterior resection (LAR) for rectal cancer, with a reported incidence of 3%-20%[1,2]. This condition is characterized by luminal narrowing at the anastomotic site, leading to clinical symptoms such as bowel obstruction, pain, bloating, and impaired quality of life[3]. The development of AS is multifactorial, involving a complex interplay of fibrosis, inflammation, anastomotic leakage, radiotherapy, ischemia, and surgical techniques[1,2]. Understanding the pathophysiology of this complication is crucial for developing effective preventive strategies and improving patient outcomes. This Minireview explores the mechanisms underlying AS, identifies key risk factors, and discusses preventive approaches.

PATHOPHYSIOLOGICAL MECHANISMS

Fibrosis and scar formation

Fibrosis is the cornerstone of AS pathogenesis, driven by an imbalance between extracellular matrix (ECM) deposition and degradation. Following rectal anastomosis, the healing process involves the activation of fibroblasts, which differentiate into myofibroblasts under the influence of transforming growth factor-beta (TGF-β), a key fibrogenic cytokine[4]. TGF-β activates the Smad signaling pathway, upregulating the expression of collagen types I and III and other ECM components, such as fibronectin and laminin[4]. This leads to excessive ECM deposition, which, if uncontrolled, forms a dense, collagen-rich scar tissue that narrows the lumen[5,6]. The imbalance between matrix metalloproteinases and their inhibitors (tissue inhibitors of metalloproteinases) further exacerbates this process by impairing ECM turnover, resulting in a fibrotic, rigid anastomotic segment[7]. Studies have shown that in patients with a history of neoadjuvant therapy, the anastomotic site exhibits increased expression of collagen and reduced elastic fibers, leading to a loss of bowel wall compliance and subsequent stricture formation[8]. Additionally, the activation of the platelet-derived growth factor (PDGF) pathway contributes to fibroblast proliferation and migration, further amplifying the fibrotic response[9]. The chronicity of this fibrotic process is often perpetuated by hypoxia and oxidative stress at the anastomotic site, which sustain TGF-β signaling and collagen deposition[10]. Fibrosis, therefore, is the primary pathophysiological driver of AS, with its severity directly correlating with the degree of luminal narrowing[5].

Role of inflammation

Inflammation is a critical mediator in the pathogenesis of AS, acting as both a necessary component of healing and a driver of pathological fibrosis when dysregulated. The early postoperative inflammatory response involves the infiltration of neutrophils and macrophages, which release pro-inflammatory cytokines such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α)[4]. These cytokines stimulate fibroblast proliferation and collagen synthesis, initiating tissue repair[4]. However, chronic or excessive inflammation—often triggered by infection, anastomotic leakage, or radiation injury—leads to a sustained activation of myofibroblasts and overproduction of ECM components[7]. This chronic inflammatory state is characterized by the infiltration of lymphocytes and plasma cells, which perpetuate the release of fibrogenic mediators like TGF-β and connective tissue growth factor (CTGF)[11]. Histopathological analyses of ASs often reveal dense inflammatory infiltrates that drive fibrotic foci, with increased expression of nuclear factor-kappa B (NF-κB), a transcription factor that amplifies the inflammatory response[12]. Moreover, the presence of oxidative stress, marked by elevated levels of reactive oxygen species (ROS), further exacerbates inflammation by activating redox-sensitive pathways such as the mitogen-activated protein kinase (MAPK) pathway, which promotes fibroblast activation and collagen deposition[13]. Factors that prolong inflammation, such as radiation-induced tissue damage or localized infection, accelerate the injury-inflammation-fibrosis cycle, significantly contributing to stricture formation[7].

Anastomotic leakage

Anastomotic leakage is a pivotal risk factor for stricture development, acting as a trigger for both inflammation and fibrosis. Leakage occurs when the anastomotic suture line fails, allowing bowel contents to escape into the surrounding

tissues, leading to localized infection, abscess formation, and a robust inflammatory response[2]. This inflammatory cascade involves the release of IL-1, IL-6, and TNF-α, which recruit immune cells and activate fibroblasts, promoting granulation tissue formation and excessive fibrosis[4]. The resulting fibrotic response creates a dense scar ring that narrows the lumen[2]. Studies have shown that anastomotic leakage significantly increases the risk of stricture, with one analysis reporting a 3.7-fold increased risk in patients with leakage[2]. The inflammatory environment created by leakage also upregulates TGF-β and CTGF, perpetuating the fibrotic process[11]. Furthermore, leakage-induced hypoxia at the anastomotic site activates hypoxia-inducible factor-1α (HIF-1α), which enhances the expression of vascular endothelial growth factor (VEGF) and other pro-fibrotic factors, further contributing to scar formation[14]. A post-hoc analysis of a randomized trial confirmed that clinical anastomotic leakage independently contributes to stricture development[15]. Thus, anastomotic leakage plays a dual role in stricture pathogenesis: It initiates inflammation and promotes scar formation, both of which contribute to luminal narrowing.

Impact of radiotherapy

Neoadjuvant radiotherapy, commonly used in rectal cancer treatment, significantly contributes to AS by inducing both acute and chronic tissue damage. Radiation causes microvascular injury, including endarteritis obliterans, which impairs blood flow to the anastomotic site and leads to tissue hypoxia[8]. This hypoxic environment triggers the release of ROS and pro-inflammatory cytokines, resulting in mucosal edema, ulceration, and chronic inflammation[8]. Over time, these subacute changes progress to fibrosis as fibroblasts are activated in response to tissue injury[8]. Radiation also upregulates TGF-β and PDGF, which promote collagen deposition and ECM remodeling[9]. Additionally, radiation-induced DNA damage in endothelial cells and fibroblasts leads to the activation of the p53 pathway, which further enhances fibrogenesis by upregulating pro-fibrotic genes[16]. Clinical studies have shown that preoperative radiotherapy increases the risk of AS, with a meta-analysis reporting a 2.3-fold higher likelihood in irradiated patients compared to non-irradiated patients[2]. Multivariate analyses indicate that radiotherapy contributes to stricture risk both directly, through its fibrogenic effects, and indirectly, by increasing the incidence of anastomotic leakage[15]. The chronic nature of radiation-induced damage, often manifesting months after treatment, underscores its role as a significant contributor to stricture pathophysiology.

Ischemic factors

Adequate blood supply is essential for anastomotic healing, and ischemia is a key contributor to stricture formation. Insufficient perfusion at the anastomotic site, often resulting from high ligation of the inferior mesenteric artery or excessive tension due to inadequate mobilization of the colon, leads to tissue hypoxia and necrosis[17]. Hypoxia activates HIF-1α, which upregulates pro-inflammatory and pro-fibrotic pathways, including the TGF-β/Smad signaling cascade and VEGF expression[14]. This results in impaired healing, with the anastomotic site undergoing a thin, fibrotic repair process that is prone to contraction[6]. Subclinical leaks may also develop in ischemic conditions, further promoting inflammation and fibrosis[6]. The ischemic environment also induces oxidative stress, with elevated ROS levels activating the MAPK and NF-κB pathways, which enhance fibroblast activation and collagen deposition[13]. A case series demonstrated that early subclinical ischemia following LAR can lead to long-segment fibrotic strictures months later[6]. Ischemia alone can initiate a cascade of inflammation and secondary fibrosis, significantly contributing to stricture formation [7]. The preservation of blood supply, such as maintaining the left colic artery when feasible, is therefore critical to preventing ischemic complications and subsequent stricture development[17].

Risk factors and surgical techniques

Several surgical and patient-related factors influence the risk of AS. The level of anastomosis is a critical determinant: Very low (rectoanal or intersphincteric) anastomoses are more prone to stricture than more proximal ones due to the narrower distal segment, limited vascularity, and frequent use of hand-sewn techniques[2]. Double-stapling techniques using mechanical staplers provide a standardized suture line and better luminal calibration, reducing stricture risk compared to hand-sewn anastomoses[2]. However, the impact of stapler diameter remains debated; a recent retrospective study found no significant difference in stricture incidence between 29 mm and 31 mm staplers[18]. Surgeon experience, technical errors (*e.g.*, incomplete stapler closure or mucosal folds), and the presence of a diverting stoma also influence stricture risk. A meta-analysis reported a threefold increased risk of stricture in patients with a diverting stoma, likely due to the lack of luminal passage and the higher baseline risk in these patients[2]. Systemic factors such as male gender, advanced age, obesity, smoking, diabetes, and high body mass index further exacerbate stricture risk by impairing wound healing[2] (Table 1).

PREVENTIVE STRATEGIES

Preventing AS requires a multifaceted approach targeting the underlying mechanisms and risk factors. Optimizing surgical techniques is paramount: Ensuring adequate blood supply by preserving the left colic artery when possible and avoiding excessive tension at the anastomotic site can reduce ischemic complications[17]. The use of double-stapling techniques with appropriately sized staplers can minimize technical errors and ensure a uniform anastomosis[2]. Minimizing the use of neoadjuvant radiotherapy, or tailoring its application to high-risk cases, may reduce radiation-induced fibrosis and leakage[15]. Early detection and management of anastomotic leakage through vigilant postoperative monitoring can mitigate the inflammatory and fibrotic cascades[2]. Additionally, addressing patient-related risk factors—such as smoking cessation, glycemic control in diabetics, and weight management—can improve wound healing and

Table 1 Key pathophysiological mechanisms, risk factors, and preventive strategies for anastomotic stricture following rectal anastomosis

Category	Details
Pathophysiological mechanisms	
Fibrosis[4,5,7,9,11]	Excessive collagen deposition (types I and III) driven by TGF-β/Smad signaling, myofibroblast activation, and imbalance of MMPs/TIMPs. Leads to dense scar tissue and luminal narrowing
Inflammation[4,7,12,13]	Dysregulated inflammatory response with IL-1, IL-6, TNF-α, and NF-κB activation. Chronic inflammation promotes fibroblast proliferation and ECM overproduction
Anastomotic leakage[2,4,11,14,15]	Leakage triggers robust inflammation (IL-1, IL-6, TNF-α) and fibrosis *via* TGF-β/CTGF. Hypoxia activates HIF-1α, enhancing pro-fibrotic pathways
Radiotherapy[2,8,9,15,16]	Radiation-induced microvascular injury (endarteritis obliterans) causes hypoxia, ROS production, and TGF-β/PDGF upregulation, leading to chronic fibrosis
Ischemia[6,7,13,14,17]	Poor blood supply (*e.g.*, due to high ligation of inferior mesenteric artery) causes hypoxia, activating HIF-1α and MAPK/NF-κB pathways, resulting in fibrotic repair
Risk factors	
Surgical factors[2,18]	Low anastomosis level (rectoanal/intersphincteric), hand-sewn *vs* double-stapling techniques, diverting stoma, technical errors (*e.g.*, incomplete stapler closure)
Patient-related factors[2]	Male gender, advanced age, obesity, smoking, diabetes, high BMI impair wound healing and increase stricture risk
Preventive strategies	
Surgical techniques[2,17,18]	Preserve left colic artery, ensure tension-free anastomosis, use double-stapling techniques with appropriate stapler size to minimize ischemia and technical errors
Perioperative care[2]	Early detection of leakage, smoking cessation, glycemic control, weight management to optimize wound healing
Emerging therapies[5]	Anti-fibrotic agents (*e.g.*, TGF-β inhibitors), novel biomaterials to modulate fibrotic response and enhance healing

TGF-β: Transforming growth factor-beta; MMPs: Matrix metalloproteinases; TIMPs: Tissue inhibitors of metalloproteinases; IL-1: Interleukin-1; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; NF-κB: Nuclear factor-kappa B; CTGF: Connective tissue growth factor; HIF-1α: Hypoxia-inducible factor-1α; PDGF: Platelet-derived growth factor; ROS: Reactive oxygen species; ECM: Extracellular matrix; BMI: Body mass index.

reduce stricture risk[2]. Future research should explore novel therapeutic strategies, such as anti-fibrotic agents, to modulate the fibrotic response in anastomotic healing[5].

CONCLUSION

AS following rectal anastomosis is a complex, multifactorial complication driven by fibrosis, inflammation, anastomotic leakage, radiotherapy, and ischemia. Excessive collagen deposition, mediated by TGF-β, PDGF, and chronic inflammation, forms the pathological basis of luminal narrowing. Risk factors such as neoadjuvant radiotherapy, low anastomotic level, and poor surgical technique further increase stricture incidence. Preventive strategies, including optimized surgical techniques, preservation of blood supply, and careful use of radiotherapy, are essential to reducing stricture risk and improving patient outcomes. Future research should focus on novel therapeutic approaches to modulate fibrotic and inflammatory responses in anastomotic healing, as well as exploring personalized medicine and novel biomaterials to further mitigate AS incidence.

FOOTNOTES

Author contributions: Yavuz A was responsible for conceptualization, methodology, data curation, writing – original draft preparation; Pehlevan-Özel H was responsible for investigation, formal analysis, visualization, writing – review & editing; Tez M was responsible for supervision, project administration, validation, writing – review & editing; all authors have read and approved the final manuscript.

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REFERENCES

1 **Liu Z**, Xu M, Yu Q, Song J, Lin Q, Huang S, Chen Z, Huang Y, Chi P. Fibrosis signature of anastomotic margins for predicting anastomotic stenosis in rectal cancer with neoadjuvant chemoradiotherapy and sphincter-preserving surgery. *Gastroenterol Rep (Oxf)* 2024; **12**: goae012 [*RCA*] [PMID: 38510669 DOI: 10.1093/gastro/goae012] [FullText] [Full Text(PDF)]

2 **He F**, Yang F, Chen D, Tang C, Woraikat S, Xiong J, Qian K. Risk factors for anastomotic stenosis after radical resection of rectal cancer: A systematic review and meta-analysis. *Asian J Surg* 2024; **47**: 25-34 [*RCA*] [PMID: 37704476 DOI: 10.1016/j.asjsur.2023.08.209] [FullText]

3 **Zhang MM**, Sha HC, Xue HR, Qin YF, Dong FF, Zhang L, Lyu Y, Yan XP. Treatment of anastomotic stricture after rectal cancer operation by magnetic compression technique: A case report. *World J Gastrointest Surg* 2024; **16**: 1443-1448 [*RCA*] [PMID: 38817285 DOI: 10.4240/wjgs.v16.i5.1443] [FullText] [Full Text(PDF)]

4 **Ismail MS**, Charabaty A. Management of Crohn's stricture: medical, endoscopic and surgical therapies. *Frontline Gastroenterol* 2022; **13**: 524-530 [*RCA*] [PMID: 36250181 DOI: 10.1136/flgastro-2021-101827] [FullText]

5 **Xin S**, Liu X, He C, Gao H, Wang B, Hua R, Gao L, Shang H, Sun F, Xu J. Inflammation accelerating intestinal fibrosis: from mechanism to clinic. *Eur J Med Res* 2024; **29**: 335 [*RCA*] [PMID: 38890719 DOI: 10.1186/s40001-024-01932-2] [FullText]

6 **Lim DR**, Hur H, Min BS, Baik SH, Kim NK. Colon Stricture After Ischemia Following a Robot-Assisted Ultra-Low Anterior Resection With Coloanal Anastomosis. *Ann Coloproctol* 2015; **31**: 157-162 [*RCA*] [PMID: 26361618 DOI: 10.3393/ac.2015.31.4.157] [FullText] [Full Text (PDF)]

7 **Wallace L**, Gallagher J. A delayed benign anastomotic stricture after anterior resection for sigmoid adenocarcinoma with concomitant collagenous colitis. *J Surg Case Rep* 2023; **2023**: rjad103 [*RCA*] [PMID: 36896151 DOI: 10.1093/jscr/rjad103] [FullText]

8 **Feng M**, Wang H, Zheng J, Chen Z, Kang B, Zhao Y, Yao J, Wang H, Zhuo S, Yan J. Association of Collagen Changes in Distal Anastomotic Margin and Anastomotic Stenosis after Neoadjuvant Chemoradiotherapy for Rectal Cancer. *J Am Coll Surg* 2024; **239**: 363-374 [*RCA*] [PMID: 38752618 DOI: 10.1097/XCS.0000000000001116] [FullText]

9 **Wynn TA**, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. *Nat Med* 2012; **18**: 1028-1040 [*RCA*] [PMID: 22772564 DOI: 10.1038/nm.2807] [FullText]

10 **Darby IA**, Hewitson TD. Fibroblast differentiation in wound healing and fibrosis. *Int Rev Cytol* 2007; **257**: 143-179 [*RCA*] [PMID: 17280897 DOI: 10.1016/S0074-7696(07)57004-X] [FullText]

11 **Leask A**, Abraham DJ. TGF-beta signaling and the fibrotic response. *FASEB J* 2004; **18**: 816-827 [*RCA*] [PMID: 15117886 DOI: 10.1096/fj.03-1273rev] [FullText]

12 **Lawrence T**. The nuclear factor NF-kappaB pathway in inflammation. *Cold Spring Harb Perspect Biol* 2009; **1**: a001651 [*RCA*] [PMID: 20457564 DOI: 10.1101/cshperspect.a001651] [FullText]

13 **Reuter S**, Gupta SC, Chaturvedi MM, Aggarwal BB. Oxidative stress, inflammation, and cancer: how are they linked? *Free Radic Biol Med* 2010; **49**: 1603-1616 [*RCA*] [PMID: 20840865 DOI: 10.1016/j.freeradbiomed.2010.09.006] [FullText] [Full Text(PDF)]

14 **Semenza GL**. Hypoxia-inducible factor 1 and the molecular physiology of oxygen homeostasis. *J Lab Clin Med* 1998; **131**: 207-214 [*RCA*] [PMID: 9523843 DOI: 10.1016/s0022-2143(98)90091-9] [FullText]

15 **Qin Q**, Ma T, Deng Y, Zheng J, Zhou Z, Wang H, Wang L, Wang J. Impact of Preoperative Radiotherapy on Anastomotic Leakage and Stenosis After Rectal Cancer Resection: Post Hoc Analysis of a Randomized Controlled Trial. *Dis Colon Rectum* 2016; **59**: 934-942 [*RCA*] [PMID: 27602924 DOI: 10.1097/DCR.0000000000000665] [FullText]

16 **Hauer-Jensen M**, Wang J, Denham JW. Bowel injury: current and evolving management strategies. *Semin Radiat Oncol* 2003; **13**: 357-371 [*RCA*] [PMID: 12903023 DOI: 10.1016/s1053-4296(03)00032-8] [FullText]

17 **Doran SLF**, Digby MG, Green SV, Kelty CJ, Tamhankar AP. Risk factors for and treatment of anastomotic strictures after Ivor Lewis esophagectomy. *Surg Endosc* 2024; **38**: 6771-6777 [*RCA*] [PMID: 39160303 DOI: 10.1007/s00464-024-11150-w] [FullText]

18 **Behboudi B**, Ahmadi-Tafti SM, Hosseini SA, Tadbir-Vajargah K, Fazeli MS, Hadizadeh A, Poopak A, Keramati MR, Kazemeini A, Ayati A, Yousefi-Koma H. The impact of circular stapler size on the risk of anastomotic stricture following total mesorectal excision in rectal cancer patients: A retrospective cross-sectional study. *Health Sci Rep* 2023; **6**: e1658 [*RCA*] [PMID: 37916143 DOI: 10.1002/hsr2.1658] [FullText]

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MINIREVIEWS

Hepatobiliary fascioliasis: A neglected re-emerging threat, its diagnostic and management challenges

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Abstract

Hepatobiliary fascioliasis is a neglected but re-emerging parasitic disease caused by *Fasciola hepatica*. Humans become infected by consuming contaminated water or aquatic plants, allowing the parasite to enter the digestive tract. From there, immature flukes penetrate the intestinal wall and migrate through the liver, triggering inflammation, fibrosis, and biliary complications. Over time, this can lead to cholangitis, biliary obstruction, and long-term liver damage. Due to its vague clinical symptoms and the limitations of current diagnostic methods, fascioliasis could be easily missed. Stool analysis is still used to detect eggs in diagnosis. However, this method is unreliable due to the inconsistency of the egg shedding. Also, serological tests are often linked to false positives due to the cross-reactions with other parasites. Imaging techniques such as ultrasound, computed tomography, and magnetic resonance imaging can reveal its complications, especially in the biliary phase, yet this is not specific. Molecular tests like polymerase chain reaction (PCR) have higher sensitivity and specificity and allow earlier diagnosis, but they are still not widely available, especially in low-resource settings. Triclabendazole is the only recommended medical treatment, yet it is not widely available. In addition, the emerging reports of resistance represent a potential threat in managing this infection. Other modalities could be needed in addition to triclabendazole, such as endoscopic retrograde cholangiopancreatography in patients with biliary complications. All the previously mentioned challenges necessitate the urgent need to make the newly developed diagnostic methods, such as PCR, available, especially in areas where fascioliasis is endemic. Additionally, new medical treatments and therapeutic options should be considered to provide a second line of management, particularly in light of emerging

reports of resistance.

Key Words: Fascioliasis; Triclabendazole; Hepatobiliary; Parasitic infections; Flukes

Core Tip: In this review article, we discuss how hepatobiliary fascioliasis, a neglected parasitic disease caused by Fasciola hepatica. Current diagnostic methods, such as stool analysis and serological tests, are unreliable and often linked to false positives. Molecular tests like polymerase chain reaction are not widely available, and new treatments and therapeutic options are needed.

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INTRODUCTION

Fascioliasis is a zoonotic parasitic infection caused by trematodes of the genus *Fasciola*, specifically *Fasciola hepatica* and *Fasciola gigantica*[1]. These liver flukes, or liver "termites" as we suggest to call, primarily infest the hepatic and biliary systems of various mammalian hosts, including humans and livestock, leading to significant pathological manifestations. *Fasciola hepatica* has a global distribution colonizing the five continents, while *Fasciola gigantica* is predominantly found in Africa and Asia. Transmission occurs *via* ingestion of water or aquatic vegetation contaminated with metacercariae, the infective larval stage of the parasite[2].

Fascioliasis is prevalent in regions characterized by extensive livestock farming and the presence of freshwater ecosystems that support the life cycle of the parasite. The disease is endemic in Latin America, Africa, Asia, and parts of Europe, where human cases are frequently reported[3].

An estimated 17 million cases of fascioliasis have been reported worldwide[3,4]. A recent meta-analysis revealed that the regions exhibiting the highest prevalence rates of fascioliasis are South America (9.0%) and Africa (4.8%)[5]. The global prevalence of fascioliasis was estimated to be 4.5%[5].

Hepatobiliary fascioliasis presents substantial public health challenges due to its capacity to induce severe hepatic and biliary complications[2]. Infected individuals may develop acute or chronic forms of the disease, with symptoms including hepatic inflammation, biliary duct obstruction, cholangitis, and secondary bacterial infections[2]. Chronic manifestations can result in fibrosis, cirrhosis, and long-term hepatic[6,7].

Fascioliasis has been documented in medical and veterinary literature for centuries, with early descriptions detailing liver fluke infestations in both humans and livestock. Historically, the disease was regarded primarily as a veterinary concern; however, its significance as a human infection has gained prominence in recent decades due to advancements in diagnostic methodologies and increased epidemiological surveillance[8]. The contemporary reemergence of fascioliasis underscores the necessity for intensified research, improved public health interventions, and the development of effective control strategies aimed at mitigating transmission and disease burden[2].

The reemergence of fascioliasis as a significant public health concern is attributed to factors such as climate change, modifications in agricultural practices, expansion of irrigation systems, and dietary changes that facilitate human exposure to infective stages[2]. An increase in the number of human infections has been documented in endemic regions, particularly in nations such as Egypt, Peru, Iran, Bolivia and Ecuador where the consumption of raw aquatic plants is customary[9,10].

In this review, we aim to delineate the etiology, epidemiology, pathophysiology, diagnostic and management challenges of hepatobiliary fascioliasis, thereby informing effective public health strategies and guiding future research amid its global reemergence. Lifecycle Fasciola have a quite complex life cycle (Figure 1). In humans, fascioliasis commences with ingestion of metacercariae-contaminated vegetation, followed by excystation in the duodenum within an hour after ingestion. The immature flukes, or "termites" as we named after them, characterizing the behavior similar to termites, then penetrate the intestinal wall and appear in the abdominal cavity within two hours after ingestion, migrate to the liver within a six-day journey, migrate through the hepatic parenchyma for around six weeks feeding on hepatic parenchyma and eventually localize in the bile ducts, where they mature[2]. According to Valero *et al*[11], the prepatent period, which is the time from ingestion of infective stage-to-seeing the diagnostic stage in feces, is around 3-4 months. We observed that the liver fluke's invasion of the biliary system is analogous to termites infiltrating a tree's inner cavities. Just as termites create and occupy protective chambers within a tree-gradually compromising its structural integrity-we observed that the fluke establishes a niche within the bile ducts, progressively impairing the host's hepatic function (Figure 2). This migratory phase underpins the acute inflammatory response, while the subsequent establishment in the biliary system precipitates chronic complications. During their migration through the hepatic parenchyma, the immature flukes mechanically disrupt tissue and provoke a localized inflammatory response. This invasive process

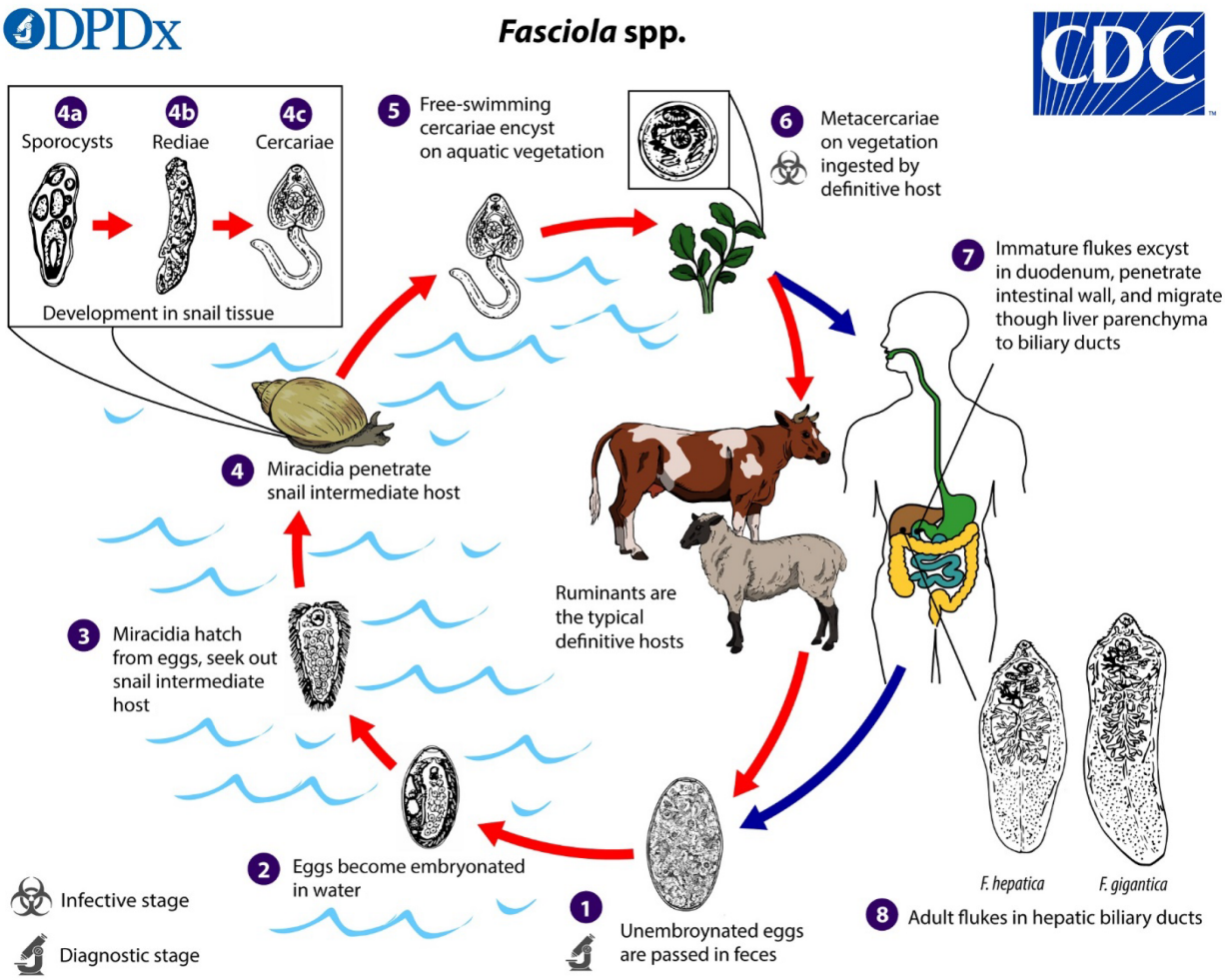

Figure 1 Life cycle of *Fasciola* spp. Immature eggs are discharged in the biliary ducts and passed in the stool (1). Eggs become embryonated in freshwater over appro-ximately 2 weeks (2); embryonated eggs release miracidia (3), which invade a suitable snail intermediate host (4). In the snail, the parasites undergo several developmental stages (sporocysts 4a, rediae 4b, and cercariae 4c). The cercariae are released from the snail (5) and encyst as metacercariae on aquatic vegetation or other substrates. Humans and other mammals become infected by ingesting metacercariae-contaminated vegetation (*e.g.*, watercress) (6). After ingestion, the metacercariae excyst in the duodenum (7) and penetrate through the intestinal wall into the peritoneal cavity. The immature flukes then migrate through the liver parenchyma into biliary ducts, where they mature into adult flukes and produce eggs (8). In humans, maturation from metacercariae into adult flukes usually takes about 3-4 months; development of *F. gigantica* may take somewhat longer than *F. hepatica*. Source: CDC-DPDx-Fascioliasis[12].

results in tissue damage characterized by focal necrosis, hemorrhage, and subsequent fibrosis. Upon reaching the biliary system, the flukes mature and establish themselves within the bile ducts. Here, their presence incites chronic irritation, promotes ductal obstruction, and induces ongoing inflammatory processes that compromise biliary integrity and hepatic function[12]. Worth noting that adult flukes can survive in the hepatobiliary system for up to 10 years[13]. This represents the basis for the chronic and relapse pictures seen in some patients[13]. Fascioliasis is characterized by a multi-phasic clinical course that reflects the dynamic interplay between parasite migration, host tissue response, and chronic biliary alterations. In the invasive (acute) phase, the initial migration of liver "termites" through the hepatic parenchyma and peritoneum precipitates significant mechanical tissue destruction[14]. This destruction elicits a pronounced inflammatory response, manifesting as systemic and localized symptoms[14]. Patients typically present with fever, right upper quadrant or epigastric pain, and gastrointestinal disturbances including anorexia, nausea, flatulence, and diarrhea[14]. Additionally, respiratory symptoms such as cough, dyspnea, and even hemoptysis may occur, accompanied by allergic phenomena such as urticaria[14]. Subsequently, during the latent phase, the parasites complete their maturation and initiate oviposition, a period often marked by minimal or subclinical symptomatology[15]. This phase may persist for months or even years, with many infections remaining undiagnosed until incidental findings emerge during family screenings or routine investigations[15]. The transition to the chronic (biliary or obstructive) phase heralds more insidious but severe clinical manifestations (Figure 3). As adult flukes establish themselves within the bile ducts, their presence incites chronic inflammation, epithelial hyperplasia, and structural remodeling of the biliary system. The resultant pathological changes include cholangitis, cholecystitis, and mechanical bile duct obstruction[7,16]. Clinically, this phase is typified by recurrent biliary colic, epigastric pain, intolerance to fatty foods, and signs of obstructive jaundice such as pruritus and right upper quadrant tenderness. Moreover, prolonged infection predisposes to gallstone formation, with the potential for secondary bacterial infections, further compounding the clinical picture[7,16]. Laboratory investigations frequently reveal eosinophilia in fascioliasis patients, particularly during the acute phase of infection. However, clinical

Figure 2 The termite's analogy illustration. We suggest calling the liver flukes the liver "termites" emphasizing the inhabitation and damage to the biliary tract and hepatic parenchyma.

manifestations during the chronic biliary phase may present without accompanying eosinophilia in some cases[17,18]. Collectively, these stages underscore the progressive and multifaceted nature of fascioliasis, where acute inflammatory damage transitions into chronic biliary pathology, with significant implications for morbidity in affected populations.

Diagnostic challenges

Traditional diagnostic approaches for fascioliasis include serological assays and stool examinations. Serological tests, such as enzyme-linked immunosorbent assays (ELISA), are commonly employed to detect antibodies against Fasciola spp. in serum, intradermal, or stool samples, offering relatively high sensitivity, particularly during the early invasive phase when eggs are not yet detectable in stool samples[19]. However, these assays may encounter specificity issues due to potential cross-reactivity with antibodies against other helminths. Conversely, stool examinations involve the microscopic identification of characteristic Fasciola eggs, serving as a direct method for diagnosing chronic infections[11]. Although stool examination provides definitive evidence of infection, its diagnostic sensitivity is notably reduced during the acute stage of the disease, and intermittent egg shedding can further complicate accurate detection. A variety of techniques, ranging from simple direct smears to various concentration methods, can be employed. Egg concentration has been successfully achieved using both flotation and sedimentation techniques. However, sedimentation techniques have demonstrated greater accuracy and sensitivity compared to flotation methods[20]. The diagnostic utility of fasciolid egg size is fraught with challenges. Traditionally, egg size thresholds-approximately 150 μm in length and 90 μm in width-have been employed to differentiate Fasciola hepatica (smaller eggs) from Fasciola gigantica (larger eggs). However, substantial variability in egg dimensions has been documented, influenced by geographical differences and the species of the definitive host, as demonstrated by studies in livestock[21,22]. The presence of intermediate forms and genetic hybrids further complicates matters, as these forms exhibit overlapping morphological characteristics that blur the conventional size distinctions. Notably, in human infections, recent computer image analysis studies have revealed that *F. hepatica* eggs tend to be larger while *F. gigantica* eggs are smaller than those observed in animals, resulting in overlapping measurement ranges that render differential diagnosis unreliable[19,23]. Consequently, reliance on classic egg size parameters in human samples may lead to erroneous diagnostic conclusions, underscoring the need for revised measurement standards in parasitological guides and clinical practice. In addition to detecting eggs through stool analyses, adults and eggs may also be identified using other invasive techniques. These include obtaining duodenal fluid, duodenal and biliary aspirates, as well as surgical procedures such as laparotomy, cholecystectomy, and sphincterotomy. Furthermore, histological examination of liver or other organ biopsy samples can also reveal their presence. Serological detection of Fasciola-specific antibodies demonstrates high diagnostic sensitivity, particularly during the acute phase when stool microscopy remains negative. ELISA platforms exhibit > 95% sensitivity and specificity, with validated targets including excretory-secretory (E/S) antigens, cathepsin proteases, and the immunodominant 27 kDa protein[24-26]. The United

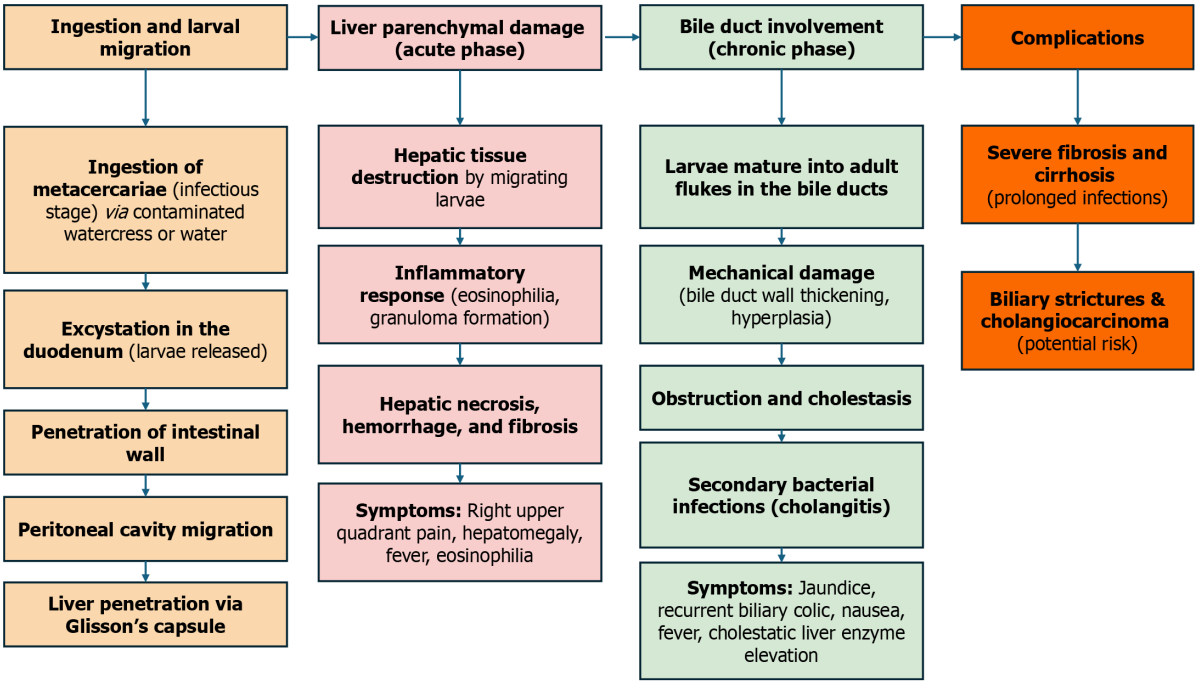

Figure 3 Flowchart of mechanisms of liver and bile duct involvement and how they lead to symptoms.

States Centers for Disease Control and Prevention provides serologic diagnosis of fascioliasis *via* an immunoblot assay detecting IgG antibodies against the recombinant FhSAP2[27,28]. Serological tests, while sensitive, may produce false-positive results in areas where multiple helminth infections are endemic, thereby limiting their specificity. Moreover, the window period between infection and seroconversion can delay diagnosis. The current diagnostic practices for fascioliasis are constrained by several limitations (Table 1)[29-33]. In response to these limitations, emerging diagnostic techniques are being developed to enhance early and accurate detection of fascioliasis. Molecular methods, particularly polymerase chain reaction (PCR) assays, have shown promise in detecting minute quantities of parasite DNA in clinical specimens, thus offering a more sensitive and specific alternative to traditional methods. PCR-based assays have been developed not only to detect Fasciola DNA with high sensitivity during early infection but also to differentiate between Fasciola hepatica and Fasciola gigantica. This differentiation is critical in endemic regions where both species-and even their hybrid forms-coexist, yet conventional clinical, pathological, stool microscopy, and immunological methods fail to distinguish them. Simple and rapid PCR-RFLP assays (using enzymes such as Ava II and Dra II) have been successfully employed to discriminate between the two pure species by targeting a conserved 28S rRNA gene fragment[34]. However, these assays, along with other PCR-based methods like duplex PCR and TaqMan real-time PCR, are limited in detecting hybrid forms due to the wide introgression capacity between the species[35]. As a result, DNA marker sequencing-targeting markers such as ITS-1, ITS-2, cox1, and nad1-remains the definitive approach for both haplotyping pure species and identifying hybridization events[35,36]. A novel isothermal PCR technique-recombinase polymerase amplification-was developed for field application in resource-limited settings. This innovative assay achieved 88% sensitivity and 100% specificity in detecting Fasciola spp. DNA in human stool samples[37]. Additionally, radiologic imaging modalities are being explored to overcome the shortcomings of traditional invasive techniques such as endoscopic retrograde cholangiopancreatography (ERCP)[38,39]. A study reported a case where Initial imaging suggested choledocholithiasis, but ERCP uncovered live Fasciola larvae in the bile duct, later confirmed by stool analysis[39]. Although ERCP has primarily been utilized for therapeutic purposes due to its invasiveness, imaging approaches-such as high-resolution ultrasonography, computed tomography (CT), and magnetic resonance imaging-could have a diagnostic potential, offering non-invasive means to visualize hepatic and biliary involvement associated with fascioliasis[17,40]. During the acute hepatic phase, contrast-enhanced CT typically demonstrates pathognomonic hypoattenuating, serpiginous subcapsular tracts. However, radiographic manifestations exhibit heterogeneity and may additionally include non-enhancing parenchymal lesions, nodular formations, hepatosplenomegaly, and regional lymphadenopathy[41].

Management challenges

The development of effective fascioliasis treatments has included the evaluation of various antiparasitic agents, as summarized in Table 2. Among these, the World Health Organization (WHO) identifies triclabendazole as the antiparasitic agent of choice for fascioliasis, demonstrating efficacy against both immature and mature stages of Fasciola spp [42]. Triclabendazole is benzimidazole drug that interferes with the parasite's β-tubulin polymerization balance leading to inhibition of protein synthesis[43]. Triclabedazole is deemed safe. Extensive global clinical experience and available safety data support the drug's favorable safety profile. However, canine studies identified potential QTc prolongation at supratherapeutic doses. Current prescribing guidelines recommend caution in patients with preexisting QTc prolongation or those concurrently using other QT-prolonging medications[44]. Standard treatment regimens with triclabendazole

Table 1 Challenges in fascioliasis diagnostics and emerging techniques

Method		Strengths	Challenges	Emerging solutions/comments
Stool analysis		Direct detection of eggs in stool samples confirms active infection	Does not allow the differentiation between *F. gigantica* and *F. hepatica* due to morphological similarities, delay in detection (only positive 3-4 months post-infection), intermittent egg output, low or absent egg shedding in light infections, chronic cases, ectopic infections, or false positives from ingested infected liver tissue	Necessitates alternative or adjunct diagnostic methods due to limited sensitivity in early and complex cases
Serology	Serological tests using excretory/secretory Antigens[29,30]	ELISA-based tests using purified or recombinant antigens (*e.g.*, cysteine proteinases produced in yeasts [29] or in *E. coli*[30]) offer high sensitivity and specificity, particularly for *F. hepatica* and *F. gigantica*	May suffer from cross-reactivity with antibodies against other helminths; delayed seroconversion in the acute phase; cannot differentiate between species in regions where both coexist	Recombinant antigens produced in yeast or *E. coli* have been successfully incorporated into ELISA protocols, improving diagnostic performance
	MM3 coproantigen-detection test[31]	Provides high sensitivity and specificity; suitable for large-scale screening, early detection in chronic infections, and monitoring treatment outcomes	Limited in its ability to quantify fluke burden on its own, which is crucial for determining appropriate treatment doses and assessing disease intensity	The use of a new preservative/diluent (CoproGuard™) has enhanced coproantigen extraction and antigen stability, potentially improving the test's diagnostic yield[32]
	Commercial *F. hepatica* IgG ELISA[26]	High sensitivity and high negative predictive value, making it useful for ruling out infection when combined with a compatible clinical history	Exhibits a low positive predictive value and lacks correlation with egg output, necessitating confirmation with additional diagnostic methods to avoid misclassification, particularly in areas with potential cross-reactive helminth infections	Considered promising for individual diagnosis and large-scale epidemiological studies, provided it is supplemented by other diagnostic tests to confirm positive results
	SeroFluke Lateral Flow Test[33]	Offers maximal specificity and sensitivity; applicable to both serum and whole-blood samples; user-friendly and suitable for point-of-care testing in both hospital settings and endemic regions	While promising, further validation is required to fully assess its performance across diverse clinical settings and to ensure its reliability in routine diagnostic practice	Represents a significant step forward in rapid, field-friendly diagnostics, potentially addressing the shortcomings of more invasive or technically demanding methods

typically involve a single or double-dose administration, tailored to parasite load and clinical severity at 10 mg/kg after a meal separated by 12-24 hours. Some studies reported cure rates between 75% and 100% using a single dose of triclabendazole 10 mg/kg[43,45,46]. Two randomized studies, with poor methodological designs, reported no difference in effectiveness between single-dose and multiple-dose regimens[45,47]. Existing evidence indicates that administering two doses of triclabendazole at 10 mg/kg may be more effective than a single-dose regimen[48]. Dosage adjustments are made based on patient-specific factors such as age, hepatic function, and the risk of reinfection, ensuring optimized therapeutic outcomes. In many endemic regions, limited access to triclabendazole poses a significant challenge. Furthermore, the emergence of triclabendazole-resistant fascioliasis is a growing concern. Extensive use in livestock has led to widespread resistance in cattle and sheep, raising the risk of transmitting resistant infections to humans[49]. Notably, treatment failures have been documented in human cases form Tureky, Peru, Portugal, Chile, and The Netherlands[50-54], with some patients continuing to shed eggs despite high-dose regimens[52]. While resistance mechanisms do not involve the same ß-tubulin mutations seen in other helminths[55,56], alterations in drug uptake and detoxification pathways are suspected[57-59]. Additionally, Nitazoxanide, 500 mg twice a day for a week in adults, has been proposed as an alternative treatment for Fasciola infections, especially in the chronic stage of infection[60]. However, evidence on its efficacy is conflicting. While some studies report success rates as high as 94%[61], others show significantly lower efficacy, with some cases failing treatment altogether[52]. Due to these inconsistencies, nitazoxanide cannot be recommended as a reliable therapeutic option. Novel antiparasitic compounds are currently under investigation, aiming to provide effective alternatives. Poor water solubility of triclabendazole may limit its organ concentration and efficacy. Flores-Ramos *et al*[62] developed a prodrug (MFR-5) that is vastly more water-soluble and stable, achieving high fasciolicidal activity in animal models. Adjunctive therapies, including surgical interventions and endoscopic procedures, play a critical role in managing complications such as biliary obstruction (Figure 4). These therapies are essential in cases where pharmacological treatment alone is insufficient to alleviate structural complications. Endoscopic intervention, particularly ERCP, can be extremely helpful in such cases, as revealed by Bahcecioglu *et al*[63]. The authors reported the use of ERCP to manage 36 patients in the biliary phase of fasciola, achieving a high success rate and safety profile. The complexity of hepatobiliary fascioliasis, particularly in advanced or refractory cases, underscores the necessity for a multidisciplinary management approach. Coordinated care involving hepatologists, infectious disease specialists, surgeons, and interventional radiologists ensures that both the parasitic infection and its systemic complications are comprehensively addressed, ultimately enhancing patient outcomes.

Table 2 Summary of antiparasitic medications, including dosages and treatment regimens

Drug	Mechanism of action	Dose	Comments
Triclabendazole	Binds to β-tubulin, inhibiting microtubule formation, leading to impaired motility and disruption of vital processes in the parasite	For patients ≥ 6 years: Two doses of 10 mg/kg administered 12 hours apart (alternatively, a single 10 mg/kg dose has been used)	Taken orally with food to improve absorption. It is the drug of choice for fascioliasis, with extensive clinical experience supporting its efficacy against both immature and mature stages. FDA-approved for use in patients aged six and older. Caution is advised in patients with preexisting QTc prolongation. Resistance has been documented in some cases
Nitazoxanide	Inhibits the pyruvate: Ferredoxin oxidoreductase (PFOR) enzyme-dependent electron transfer reaction essential for anaerobic energy metabolism	For adults: 500 mg orally twice daily for 7 days	Proposed as an alternative, especially for chronic infection. However, conflicting evidence on its efficacy (ranging from as high as 94% to as low as 36%) limits its recommendation as a reliable therapeutic option
Praziquantel	Increases calcium ion permeability in parasite membranes, causing muscle contraction and paralysis	Once considered as an alternative, praziquantel is now contraindicated for fascioliasis due to insufficient efficacy against *Fasciola* species	
Bithionol	Disrupts oxidative phosphorylation, impairing energy metabolism in parasites	A halogenated phenol formerly used as primary therapy for fascioliasis in the United States; it has been discontinued	
Emetine/Dehydroemetine	Inhibits protein synthesis by interfering with the elongation step in translation	Discontinued because of inadequate efficacy and safety issues	
Metronidazole	Undergoes reduction in anaerobic organisms to form reactive nitro radicals that damage DNA and other critical biomolecules		
Albendazole	Binds to β-tubulin, inhibiting microtubule polymerization, leading to impaired glucose uptake and energy depletion in parasites		
Niclofolan	Disrupts energy metabolism by uncoupling oxidative phosphorylation in parasite mitochondria		
Chloroquine	Inhibits DNA and RNA biosynthesis and causes degradation of ribosomes in parasites		
Hexachloro-para-xylol	Disrupts parasite metabolism through oxidative damage and interference with enzymatic processes		
Artemisinin derivatives (artesunate/artemether)	Activated by heme iron to produce free radicals that alkylate and damage parasite proteins and membranes		

Public health implications

The resurgence of fascioliasis is influenced by a confluence of environmental, socioeconomic, and ecological factors. Climate change, including increased rainfall and rising temperatures, has expanded the habitats of intermediate snail hosts, facilitating the transmission of Fasciola spp[64]. Additionally, intensified agricultural practices, such as irrigation expansion and livestock farming, contribute to environmental contamination, thereby sustaining the parasite's life cycle [65]. Socioeconomic determinants, including sanitation and restricted access to clean water, further exacerbate transmission, particularly in rural and peri-urban areas[3]. Effective control requires a multifaceted approach, treatment of livestock with anthelmintic drugs, integrating improved water resource management, snail control strategies, and agricultural policies that mitigate contamination risks[8,66]. Public awareness and education are pivotal in reducing fascioliasis transmission. Knowledge dissemination regarding risk factors, such as the consumption of raw or contaminated aquatic vegetation, is essential in high-risk communities. Community engagement and participatory education models foster local ownership of disease control strategies, ultimately improving compliance with preventive measures [10]. Additionally, environmental sustainability policies play a crucial role in controlling transmission by promoting ecological balance and sustainable agricultural practices[64]. The WHO endorses mass drug administration (MDA) to reduce human fascioliasis prevalence in endemic countries. National health authorities must implement standardized

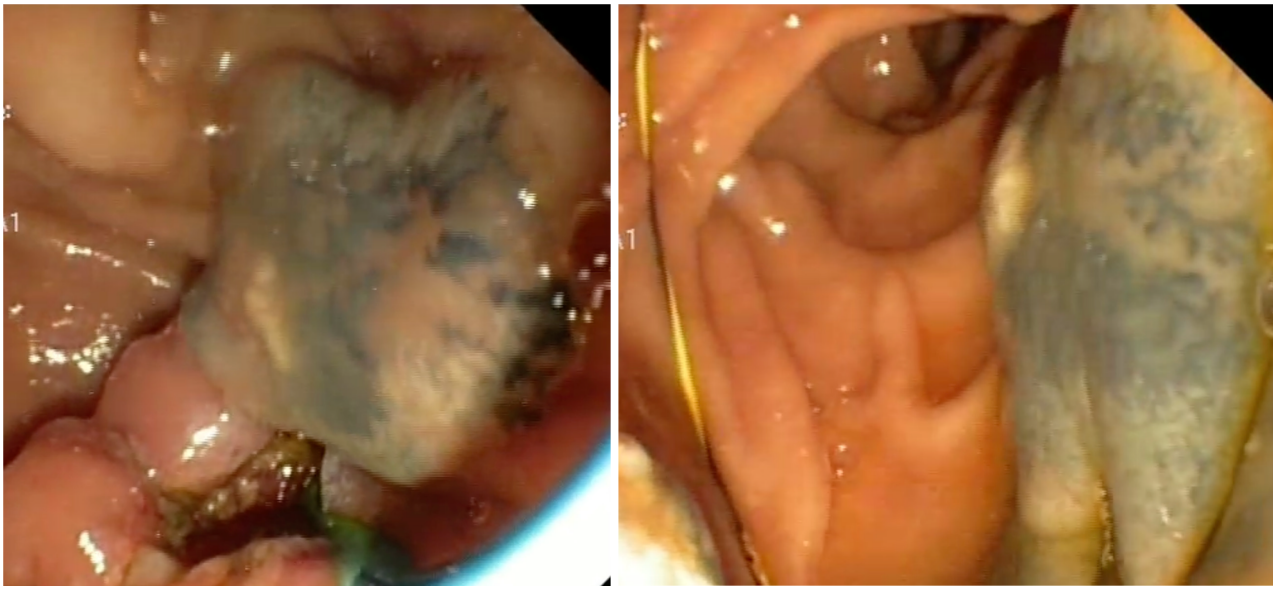

Figure 4 Viable *Fasciola* spp. Specimens retrieved from the gallbladder during endoscopic retrograde cholangiopancreatography.

treatment protocols and ensure the equitable distribution of antiparasitic drugs such as triclabendazole[67]. Bolivia, Egypt, Peru, and Vietnam have implemented various control strategies-including diagnosis-and-treatment campaigns, school-based programs, and MDA-though most are now inactive[42]. Egypt's school- and village-based screen-and-treat initiative reduced prevalence from 6% to 1% over seven years. Peru piloted a school-based MDA program in the Northern Highlands but did not expand it nationally. Bolivia sustained a decade-long MDA program near Lake Titicaca, administering fixed 250 mg triclabendazole doses annually to all residents in hyperendemic areas, regardless of age or weight[42,68,69].

CONCLUSION

Fascioliasis is a globally neglected zoonotic disease with significant health impacts. Its complex pathophysiology complicates diagnosis and management, yet timely treatment remains essential. Triclabendazole is the only recommended drug, but emerging resistance threatens control efforts.

To combat fascioliasis, investment in research, diagnostics, and alternative treatments is crucial. Strengthening global health policies, public health infrastructure, and interdisciplinary collaboration will be key to reducing transmission and disease burden. A comprehensive multi-disciplinary, sustained approach is necessary to prevent its reemergence in endemic regions.

FOOTNOTES

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REFERENCES

1 **Webb CM**, Cabada MM. Recent developments in the epidemiology, diagnosis, and treatment of Fasciola infection. *Curr Opin Infect Dis* 2018; **31**: 409-414 [*RCA*] [PMID: 30113327 DOI: 10.1097/QCO.0000000000000482] [FullText]
2 **Mas-Coma S**, Valero MA, Bargues MD. Fascioliasis. *Adv Exp Med Biol* 2019; **1154**: 71-103 [*RCA*] [PMID: 31297760 DOI: 10.1007/978-3-030-18616-6_4] [FullText]
3 **Keiser J**, Utzinger J. Food-borne trematodiases. *Clin Microbiol Rev* 2009; **22**: 466-483 [*RCA*] [PMID: 19597009 DOI: 10.1128/CMR.00012-09] [FullText]
4 **Fürst T**, Keiser J, Utzinger J. Global burden of human food-borne trematodiasis: a systematic review and meta-analysis. *Lancet Infect Dis* 2012; **12**: 210-221 [*RCA*] [PMID: 22108757 DOI: 10.1016/S1473-3099(11)70294-8] [FullText]
5 **Rosas-Hostos Infantes LR**, Paredes Yataco GA, Ortiz-Martínez Y, Mayer T, Terashima A, Franco-Paredes C, Gonzalez-Diaz E, Rodriguez-Morales AJ, Bonilla-Aldana DK, Vargas Barahona L, Grimshaw AA, Chastain DB, Sillau S, Marcos LA, Henao-Martínez AF. The global prevalence of human fascioliasis: a systematic review and meta-analysis. *Ther Adv Infect Dis* 2023; **10**: 20499361231185413 [*RCA*] [PMID: 37434654 DOI: 10.1177/20499361231185413] [FullText] [Full Text(PDF)]
6 **Marcos LA**, Terashima A, Yi P, Andrade R, Cubero FJ, Albanis E, Gotuzzo E, Espinoza JR, Friedman SL. Mechanisms of liver fibrosis associated with experimental Fasciola hepatica infection: roles of Fas2 proteinase and hepatic stellate cell activation. *J Parasitol* 2011; **97**: 82-87 [*RCA*] [PMID: 21348611 DOI: 10.1645/GE-2420.1] [FullText]
7 **Marcos LA**, Bussalleu A, Terashima A, Espinoza JR. Detection of antibodies against Fasciola hepatica in cirrhotic patients from Peru. *J Helminthol* 2009; **83**: 23-26 [*RCA*] [PMID: 18817587 DOI: 10.1017/S0022149X08067205] [FullText]
8 **Torgerson PR**, Macpherson CN. The socioeconomic burden of parasitic zoonoses: global trends. *Vet Parasitol* 2011; **182**: 79-95 [*RCA*] [PMID: 21862222 DOI: 10.1016/j.vetpar.2011.07.017] [FullText]
9 **Lopez M**, White AC Jr, Cabada MM. Burden of Fasciola hepatica Infection among children from Paucartambo in Cusco, Peru. *Am J Trop Med Hyg* 2012; **86**: 481-485 [*RCA*] [PMID: 22403322 DOI: 10.4269/ajtmh.2012.11-0448] [FullText]
10 **Haseeb AN**, el-Shazly AM, Arafa MA, Morsy AT. A review on fascioliasis in Egypt. *J Egypt Soc Parasitol* 2002; **32**: 317-354 [*RCA*] [PMID: 12049266] [FullText]
11 **Valero MA**, De Renzi M, Panova M, Garcia-Bodelon MA, Periago MV, Ordoñez D, Mas-Coma S. Crowding effect on adult growth, pre-patent period and egg shedding of Fasciola hepatica. *Parasitology* 2006; **133**: 453-463 [*RCA*] [PMID: 16817992 DOI: 10.1017/S003118200600059X] [FullText]
12 **Williams EM**. Centers for disease control and prevention. Encyclopedia of Food Controversies and the Law. 2010 [cited 2025 Mar 23]. Available from: https://www.cdc.gov/dpdx/fascioliasis/index.html
13 **Brown JD**. Human Fascioliasis (Liver Fluke Disease) in Hawai'i: Case Report and Review of Human Fascioliasis Acquired in the United States. *Hawaii J Health Soc Welf* 2021; **80**: 212-217 [*RCA*] [PMID: 34522889] [FullText]
14 **Chen MG**, Mott KE. Progress in assessment of morbidity due to Schistosoma haematobium infection: a review of recent literature. *Trop Dis Bull* 1989; **86**: R2-56
15 **Arjona R**, Riancho JA, Aguado JM, Salesa R, González-Macías J. Fascioliasis in developed countries: a review of classic and aberrant forms of the disease. *Medicine (Baltimore)* 1995; **74**: 13-23 [*RCA*] [PMID: 7837967 DOI: 10.1097/00005792-199501000-00002] [FullText]
16 **Marcos Raymundo LA**, Maco Flores V, Terashima Iwashita A, Samalvides Cuba F, Gotuzzo Herencia E. [Clinical characteristics of chronic infection by Fasciola hepatica in children]. *Rev Gastroenterol Peru* 2002; **22**: 228-233 [*RCA*] [PMID: 12378217] [FullText]
17 **Kaya M**, Beştaş R, Cetin S. Clinical presentation and management of Fasciola hepatica infection: single-center experience. *World J Gastroenterol* 2011; **17**: 4899-4904 [*RCA*] [PMID: 22171131 DOI: 10.3748/wjg.v17.i44.4899] [FullText] [Full Text(PDF)]
18 **Alvarez Rojas CA**, Jex AR, Gasser RB, Scheerlinck JP. Techniques for the diagnosis of Fasciola infections in animals: room for improvement. *Adv Parasitol* 2014; **85**: 65-107 [*RCA*] [PMID: 24928180 DOI: 10.1016/B978-0-12-800182-0.00002-7] [FullText]
19 **Mas-Coma S**, Bargues MD, Valero MA. Diagnosis of human fascioliasis by stool and blood techniques: update for the present global scenario. *Parasitology* 2014; **141**: 1918-1946 [*RCA*] [PMID: 25077569 DOI: 10.1017/S0031182014000869] [FullText]
20 **Esteban JG**, Bargues MD, Mas-Coma S. Geographical distribution, diagnosis and treatment of human fascioliasis: a review. In: Research and Reviews in Parasitology. 1998. Available from: https://api.semanticscholar.org/CorpusID:55010500
21 **Valero MA**, Darce NA, Panova M, Mas-Coma S. Relationships between host species and morphometric patterns in Fasciola hepatica adults and eggs from the northern Bolivian Altiplano hyperendemic region. *Vet Parasitol* 2001; **102**: 85-100 [*RCA*] [PMID: 11705655 DOI: 10.1016/s0304-4017(01)00499-x] [FullText]
22 **Recep T**. Yumurta Boyutlarina Göre Fasciola Gigantica Ile Fasciola Hepatica'nin Ayirimi Üzerinde Araştirmalar. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 1984; **31**: 1 [DOI: 10.1501/vetfak_0000000960] [FullText]
23 **Mas-Coma S**, Agramunt VH, Valero MA. Neurological and ocular fascioliasis in humans. *Adv Parasitol* 2014; **84**: 27-149 [*RCA*] [PMID: 24480313 DOI: 10.1016/B978-0-12-800099-1.00002-8] [FullText]
24 **Khan MAH**, Ullah R, Rehman A, Rehman L, P A AS, Abidi SMA. Immunolocalization and immunodetection of the excretory/secretory (ES) antigens of Fasciola gigantica. *PLoS One* 2017; **12**: e0185870 [*RCA*] [PMID: 28973017 DOI: 10.1371/journal.pone.0185870] [FullText] [Full Text(PDF)]
25 **Sarkari B**, Khabisi SA. Immunodiagnosis of Human Fascioliasis: An Update of Concepts and Performances of the Serological Assays. *J Clin Diagn Res* 2017; **11**: OE05-OE10 [*RCA*] [PMID: 28764235 DOI: 10.7860/JCDR/2017/26066.10086] [FullText]
26 **Valero MA**, Periago MV, Pérez-Crespo I, Rodríguez E, Perteguer MJ, Gárate T, González-Barberá EM, Mas-Coma S. Assessing the validity of an ELISA test for the serological diagnosis of human fascioliasis in different epidemiological situations. *Trop Med Int Health* 2012; **17**: 630-636 [*RCA*] [PMID: 22413850 DOI: 10.1111/j.1365-3156.2012.02964.x] [FullText]
27 **Aghamolaei S**, Mamaghani AJ, Ashrafi K, Kazemi B, Bandehpour M, Rouhani S, Rashidi S, Tabaei SJS. Designing and Developing

Serological Test for the Diagnosis of Human Fascioliasis Using a New Recombinant Multi-epitope. *Acta Parasitol* 2024; **69**: 1005-1015 [*RCA*] [PMID: 38498251 DOI: 10.1007/s11686-024-00796-0] [FullText]

28 **Shin SH**, Hsu A, Chastain HM, Cruz LA, Elder ES, Sapp SG, McAuliffe I, Espino AM, Handali S. Development of Two FhSAP2 Recombinant-Based Assays for Immunodiagnosis of Human Chronic Fascioliasis. *Am J Trop Med Hyg* 2016; **95**: 852-855 [*RCA*] [PMID: 27549636 DOI: 10.4269/ajtmh.16-0253] [FullText]

29 **O'Neill SM**, Parkinson M, Dowd AJ, Strauss W, Angles R, Dalton JP. Short report: Immunodiagnosis of human fascioliasis using recombinant Fasciola hepatica cathepsin L1 cysteine proteinase. *Am J Trop Med Hyg* 1999; **60**: 749-751 [*RCA*] [PMID: 10344647 DOI: 10.4269/ajtmh.1999.60.749] [FullText]

30 **Carnevale S**, Rodríguez MI, Guarnera EA, Carmona C, Tanos T, Angel SO. Immunodiagnosis of fasciolosis using recombinant procathepsin L cystein proteinase. *Diagn Microbiol Infect Dis* 2001; **41**: 43-49 [*RCA*] [PMID: 11687313 DOI: 10.1016/s0732-8893(01)00288-7] [FullText]

31 **Valero MA**, Periago MV, Pérez-Crespo I, Angles R, Villegas F, Aguirre C, Strauss W, Espinoza JR, Herrera P, Terashima A, Tamayo H, Engels D, Gabrielli AF, Mas-Coma S. Field evaluation of a coproantigen detection test for fascioliasis diagnosis and surveillance in human hyperendemic areas of Andean countries. *PLoS Negl Trop Dis* 2012; **6**: e1812 [*RCA*] [PMID: 23029575 DOI: 10.1371/journal.pntd.0001812] [FullText] [Full Text(PDF)]

32 **Ubeira FM**, Muiño L, Valero MA, Periago MV, Pérez-Crespo I, Mezo M, González-Warleta M, Romarís F, Paniagua E, Cortizo S, Llovo J, M ás-Coma S. MM3-ELISA detection of Fasciola hepatica coproantigens in preserved human stool samples. *Am J Trop Med Hyg* 2009; **81**: 156-162 [*RCA*] [PMID: 19556582 DOI: 10.4269/ajtmh.2009.81.156] [FullText]

33 **Martínez-Sernández V**, Muiño L, Perteguer MJ, Gárate T, Mezo M, González-Warleta M, Muro A, Correia da Costa JM, Romarís F, Ubeira FM. Development and evaluation of a new lateral flow immunoassay for serodiagnosis of human fasciolosis. *PLoS Negl Trop Dis* 2011; **5**: e1376 [*RCA*] [PMID: 22087343 DOI: 10.1371/journal.pntd.0001376] [FullText] [Full Text(PDF)]

34 **Marcilla A**, Bargues MD, Mas-Coma S. A PCR-RFLP assay for the distinction between Fasciola hepatica and Fasciola gigantica. *Mol Cell Probes* 2002; **16**: 327-333 [*RCA*] [PMID: 12477436 DOI: 10.1006/mcpr.2002.0429] [FullText]

35 **Mas-Coma S**, Valero MA, Bargues MD. Chapter 2. Fasciola, lymnaeids and human fascioliasis, with a global overview on disease transmission, epidemiology, evolutionary genetics, molecular epidemiology and control. *Adv Parasitol* 2009; **69**: 41-146 [*RCA*] [PMID: 19622408 DOI: 10.1016/S0065-308X(09)69002-3] [FullText]

36 **Siles-Lucas M**, Becerro-Recio D, Serrat J, González-Miguel J. Fascioliasis and fasciolopsiasis: Current knowledge and future trends. *Res Vet Sci* 2021; **134**: 27-35 [*RCA*] [PMID: 33278757 DOI: 10.1016/j.rvsc.2020.10.011] [FullText]

37 **Cabada MM**, Malaga JL, Castellanos-Gonzalez A, Bagwell KA, Naeger PA, Rogers HK, Maharsi S, Mbaka M, White AC Jr. Recombinase Polymerase Amplification Compared to Real-Time Polymerase Chain Reaction Test for the Detection of Fasciola hepatica in Human Stool. *Am J Trop Med Hyg* 2017; **96**: 341-346 [*RCA*] [PMID: 27821691 DOI: 10.4269/ajtmh.16-0601] [FullText]

38 **Roy S**, Mewara A, Gupta R, Rana SS. Endoscopic Diagnosis of Biliary Fascioliasis in Non-endemic Region. *Dig Dis Sci* 2023; **68**: 3476-3478 [*RCA*] [PMID: 37498416 DOI: 10.1007/s10620-023-08045-6] [FullText]

39 **Valões R**, Bonadeo NM, de Souza Quevedo P, Duro Barp AL, Fornari F. Biliary Fascioliasis: A Scare During Endoscopic Retrograde Cholangiopancreatography. *ACG Case Rep J* 2021; **8**: e00630 [*RCA*] [PMID: 34307711 DOI: 10.14309/crj.0000000000000630] [FullText] [Full Text(PDF)]

40 **Agin M**, Kayar Y, Dertli R. Diagnosis and Treatment of Fasciola Hepatica With Endoscopic Retrograde Cholangiopancreatography in a Child Patient: Case Report. *Cureus* 2020; **12**: e10486 [*RCA*] [PMID: 33083186 DOI: 10.7759/cureus.10486] [FullText] [Full Text(PDF)]

41 **Patel NU**, Bang TJ, Dodd GD 3rd. CT findings of human Fasciola hepatica infection: case reports and review of the literature. *Clin Imaging* 2016; **40**: 251-255 [*RCA*] [PMID: 26995582 DOI: 10.1016/j.clinimag.2015.11.002] [FullText]

42 **WHO**. Report of the WHO Informal Meeting on use of triclabendazole in fascioliasis control, WHO headquarters, Geneva, Switzerland, 17–18 October 2006. [cited 2025 Mar 25]. Available from: https://www.who.int/publications/i/item/WHO-CDS-NTD-PCT-2007.1

43 **Keiser J**, Engels D, Büscher G, Utzinger J. Triclabendazole for the treatment of fascioliasis and paragonimiasis. *Expert Opin Investig Drugs* 2005; **14**: 1513-1526 [*RCA*] [PMID: 16307491 DOI: 10.1517/13543784.14.12.1513] [FullText]

44 **FDA**. FDA approved drugs labels for NDA 208711. [cited 2025 May 25]. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/appletter/2019/208711Orig1s000ltr.pdf

45 **Maco V**, Marcos L, Delgado J, Herrera J, Nestares J, Terashima A, Samalvides F, Gotuzzo E. Efficacy and tolerability of two single-day regimens of triclabendazole for fascioliasis in Peruvian children. *Rev Soc Bras Med Trop* 2015; **48**: 445-453 [*RCA*] [PMID: 26312936 DOI: 10.1590/0037-8682-0148-2015] [FullText]

46 **Villegas F**, Angles R, Barrientos R, Barrios G, Valero MA, Hamed K, Grueninger H, Ault SK, Montresor A, Engels D, Mas-Coma S, Gabrielli AF. Administration of triclabendazole is safe and effective in controlling fascioliasis in an endemic community of the Bolivian Altiplano. *PLoS Negl Trop Dis* 2012; **6**: e1720 [*RCA*] [PMID: 22880138 DOI: 10.1371/journal.pntd.0001720] [FullText] [Full Text(PDF)]

47 **Talaie H**, Emami H, Yadegarinia D, Nava-Ocampo AA, Massoud J, Azmoudeh M, Mas-Coma S. Randomized trial of a single, double and triple dose of 10 mg/kg of a human formulation of triclabendazole in patients with fascioliasis. *Clin Exp Pharmacol Physiol* 2004; **31**: 777-782 [*RCA*] [PMID: 15566392 DOI: 10.1111/j.1440-1681.2004.04093.x] [FullText]

48 **Apt W**, Aguilera X, Vega F, Miranda C, Zulantay I, Perez C, Gabor M, Apt P. Treatment of human chronic fascioliasis with triclabendazole: drug efficacy and serologic response. *Am J Trop Med Hyg* 1995; **52**: 532-535 [*RCA*] [PMID: 7611560 DOI: 10.4269/ajtmh.1995.52.532] [Full Text]

49 **Kelley JM**, Elliott TP, Beddoe T, Anderson G, Skuce P, Spithill TW. Current Threat of Triclabendazole Resistance in Fasciola hepatica. *Trends Parasitol* 2016; **32**: 458-469 [*RCA*] [PMID: 27049013 DOI: 10.1016/j.pt.2016.03.002] [FullText]

50 **Gil LC**, Díaz A, Rueda C, Martínez C, Castillo D, Apt W. [Resistant human fasciolasis: report of four patients]. *Rev Med Chil* 2014; **142**: 1330-1333 [*RCA*] [PMID: 25601119 DOI: 10.4067/S0034-98872014001000014] [FullText]

51 **Winkelhagen AJ**, Mank T, de Vries PJ, Soetekouw R. Apparent triclabendazole-resistant human Fasciola hepatica infection, the Netherlands. *Emerg Infect Dis* 2012; **18**: 1028-1029 [*RCA*] [PMID: 22607719 DOI: 10.3201/eid1806.120302] [FullText] [Full Text(PDF)]

52 **Cabada MM**, Lopez M, Cruz M, Delgado JR, Hill V, White AC Jr. Treatment Failure after Multiple Courses of Triclabendazole among Patients with Fascioliasis in Cusco, Peru: A Case Series. *PLoS Negl Trop Dis* 2016; **10**: e0004361 [*RCA*] [PMID: 26808543 DOI: 10.1371/journal.pntd.0004361] [FullText] [Full Text(PDF)]

53 **Belgin G**, S KY, H T, A ÖP, B D, A D, G Y. Partial Hepatectomy for the Resistant Fasciola Hepatica Infection in a Child. *APSP J Case Rep* 2015; **6**: 27 [*RCA*] [PMID: 26623254] [FullText] [Full Text(PDF)]

54 **Branco EA**, Ruas R, Nuak J, Sarmento A. Treatment failure after multiple courses of triclabendazole in a Portuguese patient with fascioliasis. *BMJ Case Rep* 2020; **13**: e232299 [*RCA*] [PMID: 32193176 DOI: 10.1136/bcr-2019-232299] [FullText]

55 **Fuchs MA**, Ryan LA, Chambers EL, Moore CM, Fairweather I, Trudgett A, Timson DJ, Brennan GP, Hoey EM. Differential expression of liver fluke β-tubulin isotypes at selected life cycle stages. *Int J Parasitol* 2013; **43**: 1133-1139 [*RCA*] [PMID: 24162076 DOI: 10.1016/j.ijpara.2013.08.007] [FullText]

56 **Robinson MW**, Trudgett A, Hoey EM, Fairweather I. Triclabendazole-resistant Fasciola hepatica: beta-tubulin and response to in vitro treatment with triclabendazole. *Parasitology* 2002; **124**: 325-338 [*RCA*] [PMID: 11922434 DOI: 10.1017/s003118200100124x] [FullText]

57 **Meaney M**, Savage J, Brennan GP, Hoey E, Trudgett A, Fairweather I. Increased susceptibility of a triclabendazole (TCBZ)-resistant isolate of Fasciola hepatica to TCBZ following co-incubation in vitro with the P-glycoprotein inhibitor, R(+)-verapamil. *Parasitology* 2013; **140**: 1287-1303 [*RCA*] [PMID: 23756576 DOI: 10.1017/S0031182013000759] [FullText]

58 **Elliott TP**, Spithill TW. The T687G SNP in a P-glycoprotein gene of Fasciola hepatica is not associated with resistance to triclabendazole in two resistant Australian populations. *Mol Biochem Parasitol* 2014; **198**: 45-47 [*RCA*] [PMID: 25481750 DOI: 10.1016/j.molbiopara.2014.11.006] [FullText]

59 **Robinson MW**, Lawson J, Trudgett A, Hoey EM, Fairweather I. The comparative metabolism of triclabendazole sulphoxide by triclabendazole-susceptible and triclabendazole-resistant Fasciola hepatica. *Parasitol Res* 2004; **92**: 205-210 [*RCA*] [PMID: 14652740 DOI: 10.1007/s00436-003-1003-6] [FullText]

60 **Favennec L**, Jave Ortiz J, Gargala G, Lopez Chegne N, Ayoub A, Rossignol JF. Double-blind, randomized, placebo-controlled study of nitazoxanide in the treatment of fascioliasis in adults and children from northern Peru. *Aliment Pharmacol Ther* 2003; **17**: 265-270 [*RCA*] [PMID: 12534412 DOI: 10.1046/j.1365-2036.2003.01419.x] [FullText]

61 **Zumaquero-Ríos JL**, Sarracent-Pérez J, Rojas-García R, Rojas-Rivero L, Martínez-Tovilla Y, Valero MA, Mas-Coma S. Fascioliasis and intestinal parasitoses affecting schoolchildren in Atlixco, Puebla State, Mexico: epidemiology and treatment with nitazoxanide. *PLoS Negl Trop Dis* 2013; **7**: e2553 [*RCA*] [PMID: 24278492 DOI: 10.1371/journal.pntd.0002553] [FullText] [Full Text(PDF)]

62 **Flores-Ramos M**, Ibarra-Velarde F, Jung-Cook H, Hernández-Campos A, Vera-Montenegro Y, Castillo R. Novel triclabendazole prodrug: A highly water soluble alternative for the treatment of fasciolosis. *Bioorg Med Chem Lett* 2017; **27**: 616-619 [*RCA*] [PMID: 28027870 DOI: 10.1016/j.bmcl.2016.12.004] [FullText]

63 **Bahcecioglu IH**, Tawheed A, Tunc N, Artas H, Madkour A, Cicek S, Yalniz M. Characteristics of Hepatobiliary Fascioliasis and the Role of Endoscopic Retrograde Cholangiopancreatography in Management: A Single Center Experience. *J Clin Exp Hepatol* 2024; **14**: 101476 [*RCA*] [PMID: 39113686 DOI: 10.1016/j.jceh.2024.101476] [FullText]

64 **Mas-Coma S**, Valero MA, Bargues MD. Climate change effects on trematodiases, with emphasis on zoonotic fascioliasis and schistosomiasis. *Vet Parasitol* 2009; **163**: 264-280 [*RCA*] [PMID: 19375233 DOI: 10.1016/j.vetpar.2009.03.024] [FullText]

65 **WHO**. Neglected tropical diseases: Fascioliasis. [cited 2025 Mar 25]. Available from: https://www.who.int/news-room/questions-and-answers/item/q-a-on-fascioliasis

66 **Roberts JA**, Suhardono. Approaches to the control of fasciolosis in ruminants. *Int J Parasitol* 1996; **26**: 971-981 [*RCA*] [PMID: 8923144 DOI: 10.1016/s0020-7519(96)80074-9] [FullText]

67 **Mehmood K**, Zhang H, Sabir AJ, Abbas RZ, Ijaz M, Durrani AZ, Saleem MH, Ur Rehman M, Iqbal MK, Wang Y, Ahmad HI, Abbas T, Hussain R, Ghori MT, Ali S, Khan AU, Li J. A review on epidemiology, global prevalence and economical losses of fasciolosis in ruminants. *Microb Pathog* 2017; **109**: 253-262 [*RCA*] [PMID: 28602837 DOI: 10.1016/j.micpath.2017.06.006] [FullText]

68 **McManus DP**. Recent Progress in the Development of Liver Fluke and Blood Fluke Vaccines. *Vaccines (Basel)* 2020; **8**: 553 [*RCA*] [PMID: 32971734 DOI: 10.3390/vaccines8030553] [FullText] [Full Text(PDF)]

69 **Mollinedo S**, Gutierrez P, Azurduy R, Valle F, Salas A, Mollinedo Z, Soto P, Villarroel CF, Ransom J, Lawrence R, Berman J, Soto J. Mass Drug Administration of Triclabendazole for Fasciola Hepatica in Bolivia. *Am J Trop Med Hyg* 2019; **100**: 1494-1497 [*RCA*] [PMID: 31115295 DOI: 10.4269/ajtmh.19-0060] [FullText]

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ORIGINAL ARTICLE

Prospective Study

Development and validation of a risk prediction model for gastroesophageal reflux disease: Gastroesophageal Reflux Disease Risk Scoring System

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Abstract

BACKGROUND

The rising global prevalence of gastroesophageal reflux disease (GERD) has been closely linked to lifestyle changes driven by globalization. GERD imposes a substantial public health burden, affecting quality of life and leading to potential complications. Early intervention through lifestyle modification can prevent disease onset; however, there is a lack of effective risk prediction models that emphasize primary prevention.

AIM

To develop and validate a GERD Risk Scoring System (GRSS) aimed at identifying high-risk individuals and promoting primary prevention strategies.

METHODS

A 45-item questionnaire encompassing major lifestyle and demographic risk factors was developed and validated. It was administered to healthy controls and GERD patients. Two regression models—one using continuous variables and another using categorized variables—were used to develop a computational prediction equation and a clinically applicable scoring scale. An independent validation cohort of 355 participants was used to assess model performance in terms of discrimination (C-index), calibration, sensitivity, specificity, internal

consistency (Cronbach's alpha), and test-retest reliability (intraclass correlation coefficient, Bland–Altman analysis).

RESULTS

Significant associations were observed between GERD and key lifestyle factors. The derived GRSS equation and scoring scale demonstrated strong discriminative ability, with high sensitivity and specificity. The scoring system exhibited excellent internal consistency (Cronbach's alpha) and strong test-retest reliability. The C-index indicated excellent predictive accuracy in both derivation and validation cohorts.

CONCLUSION

GRSS offers a novel and validated approach to GERD risk prediction, combining a robust equation for digital applications and a practical scale for clinical use. Its ability to accurately identify at-risk individuals supports a paradigm shift toward primary prevention, underscoring its significance in addressing the growing burden of GERD at the population level.

Key Words: Gastroesophageal reflux disease; Risk prediction; Lifestyle factors; Gastroesophageal Reflux Disease Risk Scoring System score; Logistic regression; Validation study; Questionnaire; Primary prevention; Early intervention

Core Tip: There has been a steady rise in the prevalence of gastroesophageal reflux disease (GERD) due to globalization. This study introduces the GERD Risk Scoring System (GRSS), a novel primary prevention tool for assessing GERD susceptibility based on key lifestyle and demographic factors. Using logistic lasso regression, the model demonstrated high predictive accuracy, with strong internal consistency and reliability. GRSS provides both a computational risk equation and a practical scoring scale for clinical use. By enabling early identification of at-risk individuals, GRSS facilitates targeted lifestyle modifications, supporting primary prevention and reducing the long-term burden of GERD-related complications.

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INTRODUCTION

As defined by the World Gastroenterology Organization, gastroesophageal reflux disease (GERD) is characterized by troublesome symptoms that significantly impact an individual's quality of life or injuries and complications resulting from the retrograde flow of gastric contents into the esophagus, oropharynx, and/or respiratory tract[1].

The Global Burden of Disease Study reported 783.95 million cases of GERD worldwide in 2019[2]. A survey conducted by the *Indian Society of Gastroenterology* reported that the prevalence of GERD in India ranged from 7.6% to 30%[3]. This high prevalence is a major cause for concern, as GERD leads to complications such as dysphagia, perforation, bleeding, metaplasia, and adenocarcinoma, resulting in significant morbidity and mortality[4].

The American College of Gastroenterology (ACG) recommends a multimodal approach to diagnosing GERD. This starts with a detailed medical history and clinical assessment. Empirical treatment with acid-suppressing medications can serve as a diagnostic test, with symptom improvement indicating GERD. Upper gastrointestinal endoscopy is advised for severe symptoms or to confirm esophageal issues like inflammation, erosions, ulcers, or Barrett's esophagus. Additional diagnostic tools include pondus hydrogenii (pH) monitoring, esophageal manometry, barium swallow X-ray, impedance-pH monitoring, and biopsies during endoscopy[5].

While several symptom-based scoring systems for diagnosing GERD have been developed and validated worldwide, they predominantly rely on self-reported symptoms and do not account for the lifestyle factors that significantly contribute to GERD development. For instance, the GERD-Q is a validated 6-item self-administered questionnaire that aids in diagnosis based on an individual's experienced symptoms[6]. However, at the time of this publication, no scoring scale had been developed to predict the risk of developing GERD.

Established lifestyle risk factors associated with the development of GERD include[7-10] poor sleep quality and quantity, elevated body mass index (BMI), older individuals, tobacco use, alcohol consumption, dietary habits, and inadequate fiber content in the diet[11-13].

Our study aims to develop and validate a questionnaire based on risk factors to create a scoring scale to predict the risk of developing GERD. This tool can be used in the general population to identify high-risk populations so that early intervention in the form of lifestyle modifications can be advised to prevent the development of the disease and its complications.

MATERIALS AND METHODS

Study population

We designed a prospective case-control study following the principles outlined in the Helsinki Declaration. All participants in the study signed written informed consent forms. The participants in this study were recruited from the Outpatient Department of our institution.

The sample size, totaling 321 individuals (107 cases and 214 controls), was calculated using the formula described by Kelsey *et al*[14]. It is rounded off to the nearest ten (110 cases + 220 controls) for ease of calculation. The detailed sample size calculation can be found in the Supplementary material.

After explaining the study protocol to the participants, we obtained a thorough clinical history and conducted a comprehensive clinical examination. We followed the diagnostic guidelines recommended by the ACG to identify cases of GERD. Patients with typical GERD symptoms underwent endoscopy, and cases were confirmed based on evidence of erosive esophagitis, Los Angeles (LA) grade A or above. The control group subjects were asymptomatic, healthy volunteers whose upper gastrointestinal endoscopic findings were normal, recruited using a convenient sampling method from the general population. The exclusion criteria for both study groups were as follows: (1) Patients with peptic ulcer disease, hiatal hernia (of all types, including Sliding, Rolling, or Mixed, regardless of size), scleroderma, or eosinophilic esophagitis; (2) Patients below 18 years of age; and (3) Patients who did not consent to be a part of the study.

The participants in both study groups were interviewed, a questionnaire was administered, and data were collected. To assess the test-retest reliability of the GERD Risk Scoring System (GRSS) questionnaire, a subset of 40 study participants was selected to undergo a second interview with the questionnaire at a different time frame. During the time of the interview for data collection, based on the responses received, as a matter of goodwill, we advised the participants about their poor lifestyle and suggested modifications. After 6 months, we randomly selected 25 participants from our study population and telephonically interviewed them with the GRSS. We wanted to see, as a pilot, if there was a significant change in the GRSS scores following the lifestyle modification advice.

Questionnaire design

The GRSS questionnaire was developed as a novel tool to assess GERD risk based on lifestyle and demographic factors. Our approach involved adapting items from previously validated questionnaires, modifying them to target GERD-specific behaviors, and integrating newly created questions informed by known GERD risk factors identified through literature review. The questionnaire is divided into 6 modules, with 45 questions in total. The first module collects the demographic details of the patient. The other 5 modules concern smoking, alcoholism, sleep quality, diet (quantity of fiber), and stress.

Demographics: Patient identifying particulars are collected along with age, gender, weight, and height. The socioeconomic status of the patient is ascertained by using the modified Kuppusamy socioeconomic scale, where the income, occupation, and education of the head of the family are questioned[15].

Smoking: The questionnaire investigates whether the participant has a habit of smoking. The type of tobacco product smoked, quantity, and duration of smoking are questioned. Using these data, the participant's lifetime exposure to tobacco products is quantified in the form of pack years.

Alcoholism: The questionnaire investigates whether the participant has a habit of alcohol consumption. The type of alcohol consumed and quantity consumed per day are questioned to quantify the alcohol consumption per sitting in units. Using proven, multicentrically validated questionnaires for assessing alcohol abuse, such as the Alcohol Use Disorders Identification Test and Self-Rating of the Effects of Alcohol questionnaire as templates, we developed questions for the GRSS[16,17]. From these questions, a score ranging from 0 to 22 is assigned to each patient based on the responses obtained.

Sleep: The questionnaire assesses the quality of sleep of the participant. The questions were developed keeping self-rated, validated questionnaires such as the Sleep Quality Scale, Pittsburgh Sleep Quality Index, and Epworth Sleepiness Scale as templates[18-20]. From these questions, a score ranging from 0 to 12.5 is assigned to each patient based on the responses obtained. Higher scores imply a lower quality of sleep.

Diet: The questionnaire investigates whether the participant has adequate dietary fiber consumption in his diet. The questions were developed keeping an 18-item Fiber Screen and food frequency questionnaire as templates[21,22]. The questions were modified for South Indian food culture. From these questions, a score ranging from 1 to 27 is assigned to each patient based on the responses obtained. Additionally, questions were asked about the type of diet consumed, spice preference, and the time elapsed between the consumption of the last meal and bedtime.

Stress: The questionnaire assesses the psychological stress of the participants. The questions were developed keeping validated self-rated questionnaires such as the Perceived Stress Scale–4 and Holmes and Rahe Stress Scale as templates [23,24]. A score ranging from 0 to 10 is assigned to each patient based on the responses obtained. Higher scores imply that the participant faced greater psychological stress. Additionally, questions on the impact of stress on diet and destressing measures (if any) are included.

The final questionnaire is structured as a closed-ended questionnaire. Smoking and alcoholism questions serve as contingency questions, while the rest are either matrix questions, Likert scale questions, or multiple-choice questions. The GRSS features new questions specifically related to GERD development, which were not found in existing questionnaires.

The questionnaire can be found in Supplementary material. The questionnaire underwent validation, and a pilot study was conducted to optimize its design. A scoring system was developed for each module, and scores for each module were obtained according to a designed scoring legend. Content validity was established through expert consultation within their respective fields. We also employed confirmatory factor analysis to assess the construct validity and validate the questionnaire used. It is detailed in Supplementary material.

Statistical analysis

The data from the questionnaire were entered into Microsoft Excel 2020 (Microsoft Corporation, Redmond, United States). Statistical analysis was carried out using Statistical Package for the Social Sciences 26.0 for Windows (IBM, Armonk, NY, United States) and R version 4.3.2 software. Age and BMI were treated as continuous variables, while sleep score, stress score, and diet score were treated as ordinal variables. Smoking was categorized as non-smoker, smoker (< 5 pack years), or smoker (> 5 pack years), and alcoholism was classified into nonalcoholic, light drinker, or heavy drinker categories. Participants' livelihood was categorized as either urban or rural, and socioeconomic status was considered as categorical variables in our analysis. Participants were categorized as cases (GERD+ve) or controls (GERD-ve). Continuous variables were expressed as the mean ± SD or median with a 25-75 percent interquartile range. Categorical variables were expressed as percentages with a 95%CI. We considered P values < 0.05 as significant. To check the normal distribution of the data (normality), we used the Kolmogorov–Smirnov test and Anderson–Darling test. Descriptive analysis was performed to provide an overview of the collected data. Any missing data were excluded from the analysis to ensure the accuracy and reliability of the results.

We developed the GRSS using two distinct approaches: (1) Logistic regression with continuous variables (GRSS scores) for integration into websites and mobile applications, providing a user-friendly risk assessment tool; and (2) Logistic regression with categorical variables (GRSS scores categorized into intervals) was employed to create a tool tailored to be physician-friendly, ensuring ease of calculation and practicality in clinical settings.

Initially, Spearman rank correlation was used to explore associations between component scores and GERD status. Significant variables were then subjected to least absolute shrinkage and selection operator (LASSO) logistic regression analysis, including predictors such as age, BMI, sleep score, diet score, stress score, smoking status, and alcoholism. The 'glmnet' package in R was employed, applying L1 regularization (alpha = 1), and stability assessment involved bootstrapping with 10000 resamples. LASSO regression was selected over Ridge or Elastic Net regression because it performs both variable selection and regularization. Unlike Ridge regression, which only shrinks coefficients without eliminating predictors, LASSO can shrink some coefficients exactly to zero, thus identifying the most influential variables and excluding irrelevant ones. This property is valuable in clinical prediction modeling, where model simplicity and interpretability are essential for practical application in both digital tools and bedside use. The final model was selected based on minimized cross-validated error (lambda value), and it yielded coefficients for each resample.

Simultaneously, all factors were converted into categorical variables by dividing them into equally spaced intervals to design the scale. We created a contingency table for each factor and GERD status, conducting bivariate analysis using the two-tailed Fisher's exact test to qualitatively assess the association. The strength of the association between each factor and GERD was determined by Cramer's V (effect size).

We included significant variables from the bivariate analysis to create a LASSO logistic regression equation where the dependent variable (GERD) depends on the categorized component scores. Categorical coefficients, representing the change in log odds of 'GERD' with the presence of each category for a given predictor, were used to calculate a linear predictor. Subsequently, the probability of 'GERD' was derived from the linear predictor using the logistic function. The logistic regression coefficient β for each variable was utilized to create the scoring scale based on the regression coefficient-based scoring algorithm[25]. This algorithm was found to be superior to the risk ratio-based scoring algorithm. The coefficients were rounded off, and the scoring model was optimized for ease of assessment.

We recruited an additional 355 participants to prospectively evaluate the scoring scale, capturing GRSS scores as continuous variables. This expanded dataset also includes the original set of 330 participants. To illustrate the distribution of patients across different score intervals classified as non-GERD and GERD, we created two bar plots. Additionally, we generated a smooth curve to depict the predicted probability of GERD across various score values. The shaded area around the curve reflects the 95%CI, with dashed lines indicating the upper and lower bounds of this interval.

We used the 'rms' package in R to construct the logistic regression model ('f.score') and performed validation. C-index was employed as a metric to evaluate the model's discrimination ability. The validation process incorporates bootstrapping (B = 10000) to enhance reliability. We used the 'calibrate' function of the 'rms' package to assess how well the model's predicted probabilities aligned with the observed outcomes. A calibration plot was generated to visualize the model's performance, comparing observed and predicted probabilities.

The 'pROC' package in R was used to construct the receiver operating characteristic (ROC) curve for evaluating the model's ability to discriminate between individuals with and without GERD. Additionally, we calculated the area under the curve (AUC) to quantify the overall predictive accuracy. Sensitivity and specificity were computed to evaluate the model's classification performance.

To evaluate the test-retest reliability of the GRSS, we calculated the intraclass correlation coefficient (ICC) and used Bland–Altman plots to assess the consistency and agreement between the GRSS scores obtained from participants undergoing a second interview at a different time frame. The reliability of the questionnaire was assessed by Cronbach's alpha value. We used the Wilcoxon signed-rank test to determine if there was a significant change in the GRSS scores post 6 months. The flowchart for the statistical analysis conducted is depicted in Supplementary Figure 1.

RESULTS

We enrolled 330 patients: (1) 110 patients who had GERD; and (2) 220 healthy controls. All the patients enrolled in the study were South Asians. Out of the 110 cases included in the study, 46 participants were classified as LA grade A, 32 participants as LA grade B, 19 participants as LA grade C, and 13 participants as LA grade D.

The dataset exhibited a non-normal distribution, as indicated by the Kolmogorov–Smirnov and Anderson–Darling tests. Spearman rank correlation was used to find the association between continuous variables and GERD. The continuous variables were then converted into categorical variables by creating equally spaced intervals (code definitions are tabulated in Supplementary Table 1). A contingency table was created for each factor (rows) and GERD status (columns). The association between the factors and disease was qualitatively determined by the two-tailed Fisher's exact test. The strength of the association was calculated by effect size (Cramer's V). The results of the associations are tabulated in Table 1.

To assess potential multicollinearity among predictors, variance inflation factor (VIF) values were calculated using a linear model. The VIF values were: (1) Age = 1.45; (2) BMI = 1.90; (3) Sleep = 1.79; (4) Diet = 1.36; (5) Stress = 1.09; (6) Smoking status = 1.04; and (7) Alcohol level = 1.06. All VIF values were well below the commonly accepted threshold of 5, indicating no multicollinearity concerns.

A logistic LASSO regression was applied to continuous variables to formulate an equation, with the optimal regularization parameter (lambda) determined as 0.95 through bootstrapping 10000 datasets, enhancing generalization performance. The coefficients are tabulated in Table 2.

The equation obtained from this model is complex, as shown below, but it can be easily programmed for practical use in computational applications such as websites and mobile applications. GRSS scores = (-1.93) + (0.0112 × age) + (0.051 × BMI) + (0.0964 × sleep score) + (0.00589 × diet score) + (0.033 × stress score) + (-0.0159 × smoker category) + (-0.036 × alcohol category).

Scores were calculated for both the study participants and an additional cohort of 355 patients. Model validation yielded a C-index of 0.8884 through 100 bootstrap iterations. The ROC curve shown in Figure 1A, demonstrated an AUC of 0.9442, with a sensitivity of 0.8694 and specificity of 0.9659. The optimal cut-off value of 0.511 was determined based on the highest Youden index. Further plots and details of the model are available in the Supplementary material.

The GRSS scores can be converted into the risk of GERD by the following equation: Risk = $1/(1+e^{3.58 - 8.32 \times \text{GRSS scores}})$.

A practical scoring scale for clinical use was developed through logistic lasso regression using categorical variables. An optimal regularization parameter (lambda) of 0.95 was determined by bootstrapping (10000 datasets). The logistic regression coefficient β for each variable was used to create the scoring scale based on the regression coefficient-based scoring algorithm, which proved superior to the risk ratio-based algorithm. Coefficients were rounded and used to assign weights to each interval to create the scoring scale. The scoring scale is shown in Table 3.

Scores were calculated for both the study participants and an additional cohort of 355 patients. Model validation yielded a C-index of 0.891475 through 100 bootstrap iterations. The ROC curve shown in Figure 1B, demonstrated an AUC of 0.9457, with a sensitivity of 0.8408 and specificity of 0.9590. The optimal cut-off value of 25.5 was determined based on the highest Youden index. Further plots and details of the model are available in the Supplementary material.

The GRSS scores can be converted into the risk of GERD by the following equation: Risk = $1/(1+e^{15.57060 - 0.612528 \times \text{GRSS scores}})$. In Supplementary Table 2, we provide a translation of scores into corresponding risk percentages.

The reliability of the questionnaire is measured by using Cronbach's alpha value, which was found to be 0.850 indicating that the internal consistency is good. To assess the test-retest reliability of the GRSS questionnaire, 40 participants completed the questionnaire on two separate occasions. ICC analysis showed an ICC value of 0.836 ($P < 0.001$), indicating strong consistency between the two administrations. Bland-Altman analysis revealed a minimal mean difference and most data points within the limits of agreement, demonstrating high reliability. The plot is shown in Supplementary Figure 2. Wilcoxon signed-rank test to compare the GRSS scores at baseline and after 6 months indicated a statistically significant difference between the two time points, with a Wilcoxon W value of 252 ($P < 0.001$).

DISCUSSION

Our study involved 330 participants, comprising 110 GERD cases and 220 healthy controls, all of whom were of South Asian descent. Spearman rank correlation revealed an association between the risk factors and GERD. Conversion of continuous variables into categorical variables also revealed clear associations between these factors and GERD. Notably, age, BMI, smoking, alcoholism, sleep quality, diet, and stress showed significant correlations with GERD status. However, community and socioeconomic status exhibited weaker associations. After excluding non-significant factors, we took two approaches to building our risk prediction model. The first involved a lasso logistic regression with continuous variables, resulting in a rather complex equation suitable for programming websites and mobile apps. The second approach used categorical variables derived from equally spaced intervals, creating a practical scoring scale for physicians based on coefficients from the lasso logistic regression. Both models demonstrated excellent accuracy in predicting GERD risk when tested on an additional cohort of 355 individuals. Moreover, our questionnaire exhibited good internal consistency with a Cronbach's alpha value of 0.850. The test-retest reliability of the GRSS assessed by an ICC of 0.836 indicated the stability of GRSS scores over time. We found a significant change in GRSS scores following the lifestyle modification advice provided. These findings suggest that the GRSS could be a valuable tool for assessing GERD risk, considering key demographic and lifestyle factors, and facilitating timely intervention and management.

Table 1 Significance and strength of association for each risk factor of gastroesophageal reflux disease

Factor	Continuous variables		Categorical variables		
	Spearman 'rho'	*P* value	Fischer's test	Cramer's *v* value	Strength of association
Age	0.573	< 0.001[1]	< 0.001[1]	0.646	Strong
Body mass index	0.749	< 0.001[1]	< 0.001[1]	0.745	Strong
Smoking	-		< 0.001[1]	0.476	Relatively strong
Alcoholism	-		< 0.001[1]	0.434	Relatively strong
Sleep	0.710	< 0.001[1]	< 0.001[1]	0.642	Strong
Diet	0.492	< 0.001[1]	< 0.001[1]	0.639	Strong
Stress	0.289	< 0.001[1]	< 0.001[1]	0.727	Strong
Community[2]	-		0.129	0.0900	Very weak
Socio-economic status[2]	-		0.932	0.0465	Very weak

[1]Indicates significance.
[2]Categorical data.

Table 2 Coefficients of factors in the logistic regression model for gastroesophageal reflux disease

Factor	Coefficient β
Age	0.01123340
Body mass index	0.05075936
Smoking	-0.01586711
Alcoholism	-0.03592046
Sleep	0.09640941
Diet	0.00588875
Stress	0.03304126

To develop GRSS, we chose a case-control study design for its efficiency in comparing individuals with and without GERD. This approach facilitated the identification of risk factors and the creation of a GRSS. As there was no suitable existing questionnaire for primary prevention, we developed one in conjunction with the case-control study. Additionally, we explored isotonic regression for prediction models and attempted to enhance results through a combined LASSO Isotone regression model, but observed minimal impact.

We carried out an extensive literature review on databases such as PubMed/Medline, Scopus, EMBASE, and Google Scholar, focused on studies related to GERD risk prediction. We employed search terms such as "GERD risk prediction", "GERD questionnaire", "Gastroesophageal Reflux Disease prediction tool", and "GERD risk assessment". We found that various questionnaires are available to assess GERD. The GERD-Q is a validated tool designed to gauge the frequency and severity of GERD symptoms, and how these symptoms affect daily life[6]. Similarly, the GERD Symptom Assessment Scale evaluates the severity and frequency of GERD symptoms such as heartburn, regurgitation, chest pain, and swallowing difficulties, while also delving into their repercussions on a patient's quality of life[26]. The Reflux Disease Questionnaire is widely employed to gauge the frequency and severity of heartburn, regurgitation, and epigastric pain, giving a composite score that quantifies GERD symptom severity[27]. The Carlsson-Dent GERD Questionnaire investigates symptom presence and severity, assigning a score indicative of GERD likelihood[28]. The Quality of Life in Reflux and Dyspepsia captures the impact of GERD on a patient's quality of life, encompassing physical and emotional well-being[29]. We also found several studies that aimed to create risk prediction models for assessing Barett's esophagus[30-32]. However, we found a significant gap in studies specifically addressing GERD risk prediction, prompting our central research question: How can we strategically identify individuals at risk of developing GERD. In the realm of primary prevention, how can we tailor preventive measures using a predictive risk factor model-GRSS to curtail the onset of GERD and its related complications.

GERD is linked to various lifestyle factors, with six major ones examined in our study. Older individuals experience higher GERD prevalence due to lax lower esophageal sphincters, as noted by Euseb *et al*[33]. Increased body weight, reflected by BMI, leads to more adipocytes which secrete adiponectin and leptin, disrupting sphincter function and causing regurgitation of gastric contents[34]. Luminal nitric acid, known for its cytotoxic effects, may impair the epithelial barrier, with high-fiber foods potentially mitigating this risk[35]. Smoking lowers the resting tone of the lower esophageal

Table 3 Gastroesophageal reflux disease Risk Scoring Scale (Gastroesophageal Reflux Disease Risk Scoring System)

Factor	Parameter	Score
Age (years)	Less than 20	3
	21-40	5
	41-60	8
	61-80	11
	Greater than 81	13
Body mass index	Less than 18	3
	18-22.9	7
	23-24.9	10
	More than 25	13
Smoking	Non-smoker	0
	Smoker < 5 pack years	0.2
	Smoker > 5 pack years	0.4
Alcohol score	Non-alcoholic	0
	0–6	0·2
	7–12	1.4
Diet score	< 4	2
	4–8	1.5
	8–12	1
	> 12	0.5
Stress score	< 4.5	1
	4.5–6	2
	> 6	2.5
Sleep score	< 4	3
	4–8	6
	> 8	9

sphincter, enhances acid reflux, and reduces bicarbonate levels in saliva[10]. Alcohol consumption results in toxic byproducts like acetaldehyde, which disrupt sphincter function and acid secretion, impacting GERD[36]. Sleep disturbances and lying down exacerbate symptoms, particularly if sleep occurs within three hours of eating[37]. Stress increases catecholamine levels, worsening esophageal sphincter action and maintaining inflammatory states that exacerbate GERD symptoms[38].

From the patient's point of view, this innovative tool provided a chance for individuals to detect their vulnerability to GERD at an early stage and facilitated prompt adjustments to their lifestyle. By enabling early identification of risk and emphasizing lifestyle changes, this tool enhanced personal well-being and reduced the risk of complications associated with GERD. From adopting dietary adjustments to healthier sleep patterns and stress management, patients found that this screening tool aligned with their desire for preventive healthcare. The questionnaire was carefully designed with the patient's convenience in mind, ensuring that it was easy to use and did not take more than 8 minutes to complete, further enhancing user-friendliness.

An added advantage of the GRSS questionnaire lies in its component scores, allowing us to pinpoint specific risk factors for each individual. This precision enables us to recommend tailored lifestyle modifications to address the particular risk factor identified. By offering personalized advice, the GRSS allows individuals to make changes that directly mitigate their unique risks. This not only enhances the effectiveness of preventive measures but also ensures a more individualized and patient-centric approach to managing GERD risk. The risk factors selected in our study are primarily associated with lifestyle habits. Although we have developed the GRSS as a tool for assessing the risk of GERD development, our study did not assess whether improvements in lifestyle leading to a decrease in GRSS scores led to a reduced risk of GERD. This suggests a potential direction for future research, which could involve an intervention study to assess its clinical utility.

GRSS includes non-integer values, which may appear unconventional in clinical scoring tools. While integer scores are often preferred for their simplicity, we chose to retain non-integer scores because they better reflect clinically meaningful

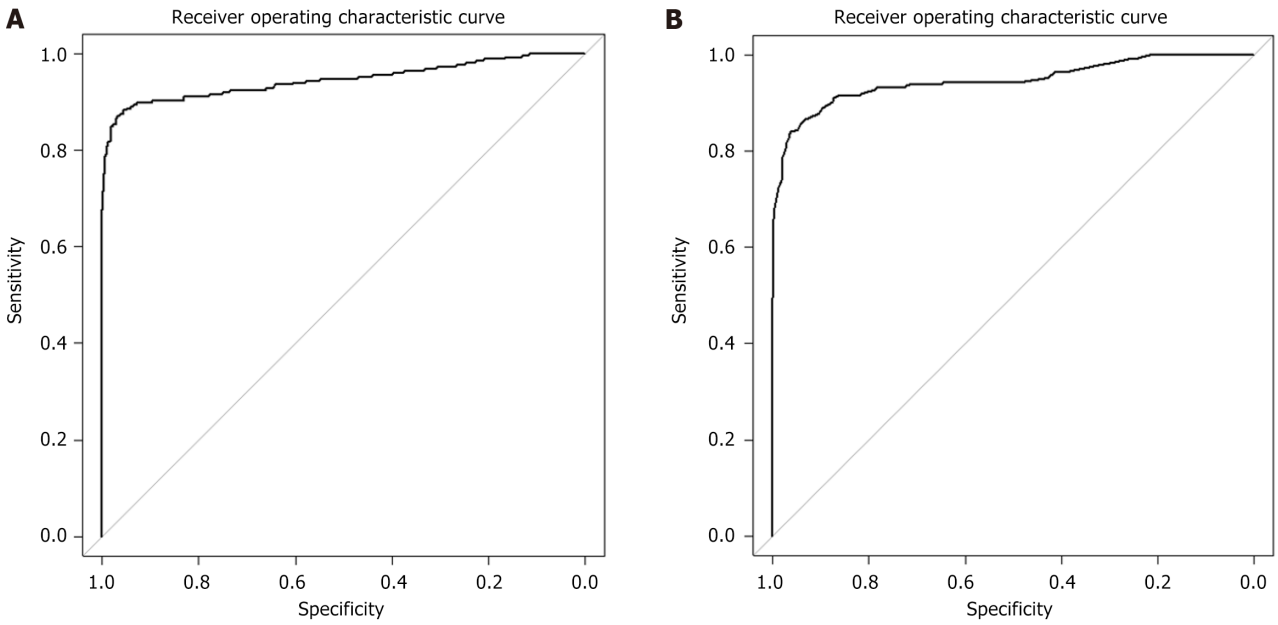

Figure 1 Receiver operating characteristic curve. A: Receiver operating characteristic (ROC) for discriminative accuracy of Gastroesophageal Reflux Disease Risk Scoring System (GRSS) modeled using continuous variables (approach 1); B: ROC curve for discriminative accuracy of GRSS modeled using categorical variables (approach 2).

risk thresholds. For instance, in the smoking module, we initially considered higher pack-year thresholds (< 50 pack years and > 50 pack years) yielding integer based scores. However, this approach proved suboptimal for clinical utility. Most young individuals in our primary prevention target group do not yet reach such high cumulative exposures. As a result, the scoring system would fail to capture early, modifiable risk in this crucial population. By lowering the threshold to < 5 pack years and > 5 pack years, we ensured that the tool remains sensitive to early smoking exposure. This adjustment resulted in non-integer scores but it preserves the lead time advantage essential to any effective screening or preventive measure.

Beyond its utility as a research instrument, the GRSS holds immense promise in clinical settings. Its user-friendly design facilitates regular and accurate GERD risk assessment by patients or healthcare workers. Clinicians should maintain a high index of suspicion for GERD in patients with higher GRSS scores and initiate appropriate interventions promptly to prevent the development of complications. While the development of the GRSS represents a significant leap forward, we acknowledge certain limitations and areas for future refinement. These include the need for further validation of the tool in multicentric studies to establish the reliability of this scoring system. The GRSS was developed and validated in a South Asian setting. Consequently, the generalizability of the questionnaire to different populations may be affected, highlighting the need for regional modifications in the questionnaire by further studies. GERD is influenced by 19 risk factors; our questionnaire focuses on the six major risk factors. While this strategic selection streamlines the questionnaire's practicality, it also limits the complete evaluation of all the risk factors for GERD. Additionally, self-reported data introduces the possibility of recall bias, which is an inherent limitation in questionnaire-based studies assessing lifestyle risk factors. However, such tools remain essential for early screening and public health utility due to their practicality and scalability. To mitigate recall bias, the GRSS questionnaire was carefully designed with objective, specific, and behaviorally anchored questions to facilitate more accurate recall and reporting. Another limitation is that, in our pilot, we assessed if there was a change in GRSS scores following unofficial lifestyle modification advice but could not assess compliance. Future research should address this aspect. We are committed to addressing these issues and continually enhancing the GRSS to meet the evolving needs of both researchers and clinicians. A visual illustration of the clinical implication of GRSS is shown in Figure 2.

CONCLUSION

In conclusion, we have developed a new novel questionnaire–GRSS that may predict the susceptibility of an individual to developing GERD in the future. Based on the GRSS scores, lifestyle modifications can be advised to the individual. GRSS proves itself on two fronts—first, with a sophisticated equation suitable for computational applications, and second, with a user-friendly scoring scale for clinicians. Both approaches showed remarkable precision in predicting GERD risk which highlighted the GRSS's potential for early intervention in clinical settings.

The substantial prevalence of GERD within our South Asian study group emphasizes the broader public health implications of GRSS. This approach focuses on primary prevention rather than the traditional secondary prevention tools such as screening, which typically identify individuals after GERD has already developed. Our method allows us to use the tool across the entire population, categorizing people into low-risk, medium-risk, and high-risk groups. Those in the low-

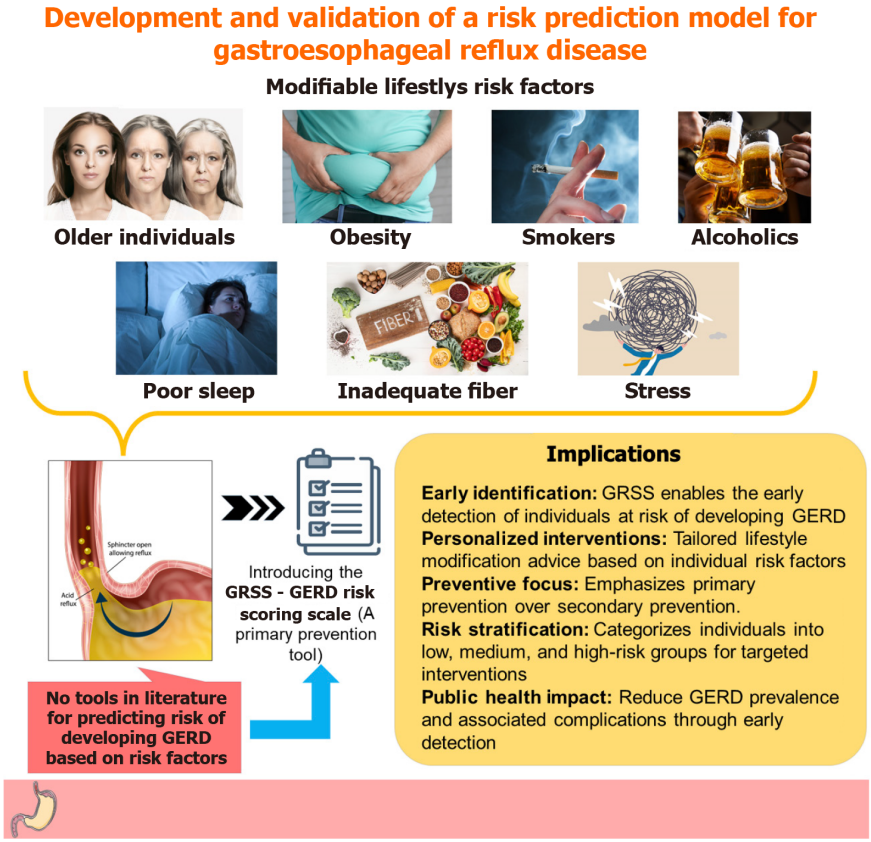

Figure 2 Graphical illustration of the clinical implications of the Gastroesophageal Reflux Disease Risk Scoring System. GERD: Gastroesophageal reflux disease; GRSS: Gastroesophageal Reflux Disease Risk Scoring System.

risk and medium-risk categories are advised on lifestyle changes to minimize the risk of GERD, while high-risk individuals undergo additional screening to check for the disease. This not only optimizes preventive strategies but also ensures more targeted and efficient healthcare interventions based on individual risk levels, marking a significant step forward in promoting overall health and well-being. While our study has inherent limitations, it serves as a stepping-stone for future research in the field of GERD.

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FOOTNOTES

Author contributions: Subramanian S was involved in conceptualization, formal analysis, writing the first draft, literature search, study design, and data collection; Sundararaju U contributed to conceptualization, formal analysis, methodology, writing the first draft, literature search, study design, and data collection; Rajakumar HK contributed to conceptualization, data curation, formal analysis, methodology, writing the first draft, data collection, analysis, and interpretation; Sathyabal VC participated in conceptualization, formal analysis, writing the first draft, literature search, and study design; Murugan A provided supervision, data analysis, and reviewed and edited the manuscript; Gnanavel P and Sathishkumar K were responsible for data analysis and reviewing and editing the manuscript; all authors had access to the data, contributed to the manuscript, and approved the final version for publication.

Institutional review board statement: This study was approved by the Institutional Ethics Committee of Government Medical College, Omandurar, Government Estate. All procedures performed in studies involving human patients were in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments.

Clinical trial registration statement: This study is not a clinical trial as defined by the International Committee of Medical Journal Editors and the World Health Organization. This study was not required to be registered as a clinical trial. The research received approval from

the local Institutional Ethics Committee.

Informed consent statement: Written informed consent for participation and publication has been obtained from the study participants.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: The data utilized in this research will be accessible upon request from Rajakumar HK but will not be publicly accessible to safeguard the confidentiality and privacy of the patients who participated. Requests for data access must specify the purpose for which the data will be utilized. In cases of data reuse, a proposal outlining the purpose, the intended usage of the data, and a letter from the department head or the institution's leadership will be mandatory. Additionally, any subsequent data generation should be communicated to the Rajakumar HK.

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REFERENCES

1 **World Gastroenterology Organisation**. World Gastroenterology Organisation Global Guidelines – GERD - Global Perspective on Gastroesophageal Reflux Disease. Available from: https://www.worldgastroenterology.org/guidelines/gastroesophageal-reflux-disease/gastroesophageal-reflux-disease-english

2 **Zhang D**, Liu S, Li Z, Wang R. Global, regional and national burden of gastroesophageal reflux disease, 1990-2019: update from the GBD 2019 study. *Ann Med* 2022; **54**: 1372-1384 [*RCA*] [PMID: 35579516 DOI: 10.1080/07853890.2022.2074535] [FullText] [Full Text(PDF)]

3 **Bhatia SJ**, Makharia GK, Abraham P, Bhat N, Kumar A, Reddy DN, Ghoshal UC, Ahuja V, Rao GV, Devadas K, Dutta AK, Jain A, Kedia S, Dama R, Kalapala R, Alvares JF, Dadhich S, Dixit VK, Goenka MK, Goswami BD, Issar SK, Leelakrishnan V, Mallath MK, Mathew P, Mathew P, Nandwani S, Pai CG, Peter L, Prasad AVS, Singh D, Sodhi JS, Sud R, Venkataraman J, Midha V, Bapaye A, Dutta U, Jain AK, Kochhar R, Puri AS, Singh SP, Shimpi L, Sood A, Wadhwa RT. Indian consensus on gastroesophageal reflux disease in adults: A position statement of the Indian Society of Gastroenterology. *Indian J Gastroenterol* 2019; **38**: 411-440 [*RCA*] [PMID: 31802441 DOI: 10.1007/s12664-019-00979-y] [FullText]

4 **Richter JE**, Rubenstein JH. Presentation and Epidemiology of Gastroesophageal Reflux Disease. *Gastroenterology* 2018; **154**: 267-276 [*RCA*] [PMID: 28780072 DOI: 10.1053/j.gastro.2017.07.045] [FullText]

5 **Katz PO**, Dunbar KB, Schnoll-Sussman FH, Greer KB, Yadlapati R, Spechler SJ. ACG Clinical Guideline for the Diagnosis and Management of Gastroesophageal Reflux Disease. *Am J Gastroenterol* 2022; **117**: 27-56 [*RCA*] [PMID: 34807007 DOI: 10.14309/ajg.0000000000001538] [FullText]

6 **Jones R**, Junghard O, Dent J, Vakil N, Halling K, Wernersson B, Lind T. Development of the GerdQ, a tool for the diagnosis and management of gastro-oesophageal reflux disease in primary care. *Aliment Pharmacol Ther* 2009; **30**: 1030-1038 [*RCA*] [PMID: 19737151 DOI: 10.1111/j.1365-2036.2009.04142.x] [FullText]

7 **Dickman R**, Green C, Fass SS, Quan SF, Dekel R, Risner-Adler S, Fass R. Relationships between sleep quality and pH monitoring findings in persons with gastroesophageal reflux disease. *J Clin Sleep Med* 2007; **3**: 505-513 [*RCA*] [PMID: 17803014 DOI: 10.5664/jcsm.26915] [Full Text]

8 **Chang P**, Friedenberg F. Obesity and GERD. *Gastroenterol Clin North Am* 2014; **43**: 161-173 [*RCA*] [PMID: 24503366 DOI: 10.1016/j.gtc.2013.11.009] [FullText]

9 **Moshkowitz M**, Horowitz N, Halpern Z, Santo E. Gastroesophageal reflux disease symptoms: prevalence, sociodemographics and treatment patterns in the adult Israeli population. *World J Gastroenterol* 2011; **17**: 1332-1335 [*RCA*] [PMID: 21455333 DOI: 10.3748/wjg.v17.i10.1332] [FullText] [Full Text(PDF)]

10 **Pandolfino JE**, Kahrilas PJ. Smoking and gastro-oesophageal reflux disease. *Eur J Gastroenterol Hepatol* 2000; **12**: 837-842 [*RCA*] [PMID: 10958210 DOI: 10.1097/00042737-200012080-00002] [FullText]

11 **Pan J**, Cen L, Chen W, Yu C, Li Y, Shen Z. Alcohol Consumption and the Risk of Gastroesophageal Reflux Disease: A Systematic Review and Meta-analysis. *Alcohol Alcohol* 2019; **54**: 62-69 [*RCA*] [PMID: 30184159 DOI: 10.1093/alcalc/agy063] [FullText]

12 **Taraszewska A**. Risk factors for gastroesophageal reflux disease symptoms related to lifestyle and diet. *Rocz Panstw Zakl Hig* 2021; **72**: 21-28 [*RCA*] [PMID: 33882662 DOI: 10.32394/rpzh.2021.0145] [FullText]

13 **Morozov S**, Isakov V, Konovalova M. Fiber-enriched diet helps to control symptoms and improves esophageal motility in patients with non-erosive gastroesophageal reflux disease. *World J Gastroenterol* 2018; **24**: 2291-2299 [*RCA*] [PMID: 29881238 DOI: 10.3748/wjg.v24.i21.2291] [FullText] [Full Text(PDF)]

14 **Kelsey JL**, Thompson WD, Evans AS. Methods in observational epidemiology. New York: Oxford University Press, 1986

15 **Wani RT**. Socioeconomic status scales-modified Kuppuswamy and Udai Pareekh's scale updated for 2019. *J Family Med Prim Care* 2019; **8**: 1846-1849 [*RCA*] [PMID: 31334143 DOI: 10.4103/jfmpc.jfmpc_288_19] [FullText] [Full Text(PDF)]

16 Alcohol use disorders identification test (AUDIT). Available from: https://auditscreen.org/

17 **Schuckit MA**, Smith TL, Danko GP, Pierson J, Hesselbrock V, Bucholz KK, Kramer J, Kuperman S, Dietiker C, Brandon R, Chan G. The ability of the Self-Rating of the Effects of Alcohol (SRE) Scale to predict alcohol-related outcomes five years later. *J Stud Alcohol Drugs* 2007; **68**: 371-378 [*RCA*] [PMID: 17446976 DOI: 10.15288/jsad.2007.68.371] [FullText]

18 **Snyder E**, Cai B, DeMuro C, Morrison MF, Ball W. A New Single-Item Sleep Quality Scale: Results of Psychometric Evaluation in Patients With Chronic Primary Insomnia and Depression. *J Clin Sleep Med* 2018; **14**: 1849-1857 [*RCA*] [PMID: 30373688 DOI: 10.5664/jcsm.7478] [FullText]

19 **Buysse DJ**, Reynolds CF 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989; **28**: 193-213 [*RCA*] [PMID: 2748771 DOI: 10.1016/0165-1781(89)90047-4] [FullText]

20 **Johns MW**. A new method for measuring daytime sleepiness: the Epworth sleepiness scale. *Sleep* 1991; **14**: 540-545 [*RCA*] [PMID: 1798888 DOI: 10.1093/sleep/14.6.540] [FullText]

21 **Rijnaarts I**, de Roos N, Zoetendal EG, de Wit N, Witteman BJM. Development and validation of the FiberScreen: A short questionnaire to screen fibre intake in adults. *J Hum Nutr Diet* 2021; **34**: 969-980 [*RCA*] [PMID: 34378249 DOI: 10.1111/jhn.12941] [FullText] [Full Text (PDF)]

22 **Neyrinck AM**, Nazare JA, Rodriguez J, Jottard R, Dib S, Sothier M, Berghe LVD, Alligier M, Alexiou H, Maquet V, Vinoy S, Bischoff SC, Walter J, Laville M, Delzenne NM. Development of a Repertoire and a Food Frequency Questionnaire for Estimating Dietary Fiber Intake Considering Prebiotics: Input from the FiberTAG Project. *Nutrients* 2020; **12**: 2824 [*RCA*] [PMID: 32942686 DOI: 10.3390/nu12092824] [Full Text] [Full Text(PDF)]

23 **Cohen S**, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* 1983; **24**: 385-396 [*RCA*] [PMID: 6668417 DOI: 10.2307/2136404] [FullText]

24 **Noone PA**. The Holmes-Rahe Stress Inventory. *Occup Med (Lond)* 2017; **67**: 581-582 [*RCA*] [PMID: 29048597 DOI: 10.1093/occmed/kqx099] [FullText]

25 **Mehta HB**, Mehta V, Girman CJ, Adhikari D, Johnson ML. Regression coefficient-based scoring system should be used to assign weights to the risk index. *J Clin Epidemiol* 2016; **79**: 22-28 [*RCA*] [PMID: 27181564 DOI: 10.1016/j.jclinepi.2016.03.031] [FullText]

26 **Rothman M**, Farup C, Stewart W, Helbers L, Zeldis J. Symptoms associated with gastroesophageal reflux disease: development of a questionnaire for use in clinical trials. *Dig Dis Sci* 2001; **46**: 1540-1549 [*RCA*] [PMID: 11478508 DOI: 10.1023/a:1010660425522] [FullText]

27 **Shaw M**, Dent J, Beebe T, Junghard O, Wiklund I, Lind T, Johnsson F. The Reflux Disease Questionnaire: a measure for assessment of treatment response in clinical trials. *Health Qual Life Outcomes* 2008; **6**: 31 [*RCA*] [PMID: 18447946 DOI: 10.1186/1477-7525-6-31] [Full Text] [Full Text(PDF)]

28 **Contreras-Omaña R**, Sánchez-Reyes O, Ángeles-Granados E. Comparison of the Carlsson-Dent and GERD-Q questionnaires for gastroesophageal reflux disease symptom detection in a general population. *Rev Gastroenterol Mex* 2017; **82**: 19-25 [*RCA*] [PMID: 27865578 DOI: 10.1016/j.rgmx.2016.05.005] [FullText]

29 **Wiklund IK**, Junghard O, Grace E, Talley NJ, Kamm M, Veldhuyzen van Zanten S, Paré P, Chiba N, Leddin DS, Bigard MA, Colin R, Schoenfeld P. Quality of Life in Reflux and Dyspepsia patients. Psychometric documentation of a new disease-specific questionnaire (QOLRAD). *Eur J Surg Suppl* 1998; 41-49 [*RCA*] [PMID: 10027672] [FullText]

30 **Rosenfeld A**, Graham DG, Jevons S, Ariza J, Hagan D, Wilson A, Lovat SJ; BEST2 study group, Sami SS, Ahmad OF, Novelli M, Rodriguez Justo M, Winstanley A, Heifetz EM, Ben-Zecharia M, Noiman U, Fitzgerald RC, Sasieni P, Lovat LB. Development and validation of a risk prediction model to diagnose Barrett's oesophagus (MARK-BE): a case-control machine learning approach. *Lancet Digit Health* 2020; **2**: E37-E48 [*RCA*] [PMID: 32133440 DOI: 10.1016/S2589-7500(19)30216-X] [FullText]

31 **Thrift AP**, Kendall BJ, Pandeya N, Vaughan TL, Whiteman DC; Study of Digestive Health. A clinical risk prediction model for Barrett esophagus. *Cancer Prev Res (Phila)* 2012; **5**: 1115-1123 [*RCA*] [PMID: 22787114 DOI: 10.1158/1940-6207.CAPR-12-0010] [FullText]

32 **Ireland CJ**, Fielder AL, Thompson SK, Laws TA, Watson DI, Esterman A. Development of a risk prediction model for Barrett's esophagus in an Australian population. *Dis Esophagus* 2017; **30**: 1-8 [*RCA*] [PMID: 28881896 DOI: 10.1093/dote/dox033] [FullText]

33 **Eusebi LH**, Ratnakumaran R, Yuan Y, Solaymani-Dodaran M, Bazzoli F, Ford AC. Global prevalence of, and risk factors for, gastro-oesophageal reflux symptoms: a meta-analysis. *Gut* 2018; **67**: 430-440 [*RCA*] [PMID: 28232473 DOI: 10.1136/gutjnl-2016-313589] [FullText]

34 **Vaishnav B**, Bamanikar A, Maske P, Reddy A, Dasgupta S. Gastroesophageal Reflux Disease and its Association with Body Mass Index: Clinical and Endoscopic Study. *J Clin Diagn Res* 2017; **11**: OC01-OC04 [*RCA*] [PMID: 28571180 DOI: 10.7860/JCDR/2017/24151.9562] [FullText]

35 **Iijima K**, Shimosegawa T. Involvement of luminal nitric oxide in the pathogenesis of the gastroesophageal reflux disease spectrum. *J Gastroenterol Hepatol* 2014; **29**: 898-905 [*RCA*] [PMID: 24863184 DOI: 10.1111/jgh.12548] [FullText]

36 **Chen SH**, Wang JW, Li YM. Is alcohol consumption associated with gastroesophageal reflux disease? *J Zhejiang Univ Sci B* 2010; **11**: 423-428 [*RCA*] [PMID: 20506572 DOI: 10.1631/jzus.B1000013] [FullText]

37 **Jung HK**, Choung RS, Talley NJ. Gastroesophageal reflux disease and sleep disorders: evidence for a causal link and therapeutic implications. *J Neurogastroenterol Motil* 2010; **16**: 22-29 [*RCA*] [PMID: 20535322 DOI: 10.5056/jnm.2010.16.1.22] [FullText] [Full Text(PDF)]

38 **Song EM**, Jung HK, Jung JM. The association between reflux esophagitis and psychosocial stress. *Dig Dis Sci* 2013; **58**: 471-477 [*RCA*] [PMID: 23001402 DOI: 10.1007/s10620-012-2377-z] [FullText] [Full Text(PDF)]

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