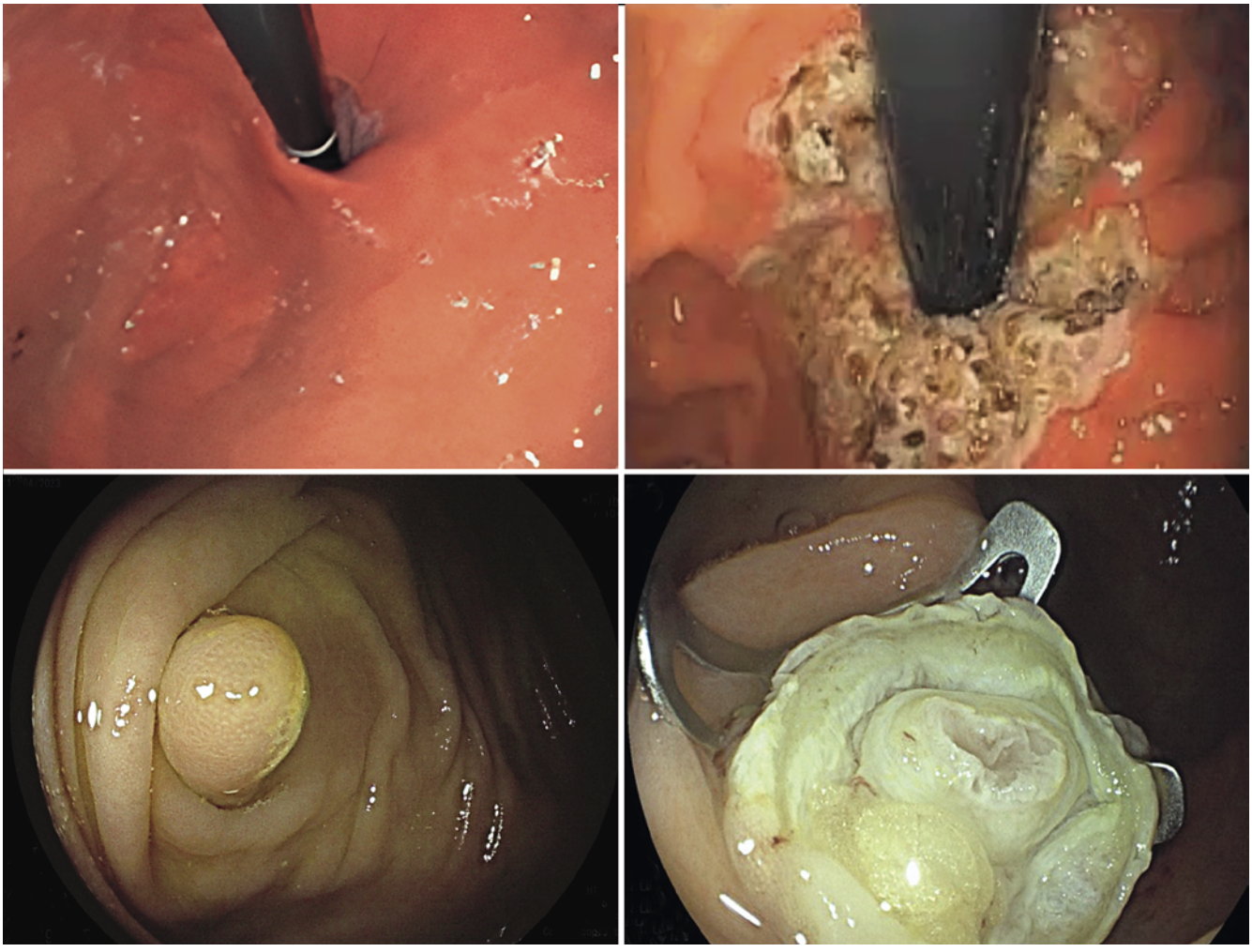
Portuguese Journal of Gastroenterology

31 | 5 | 24



Highlights in this issue:

Review article: Portuguese Pancreatic Club Perspective on the Surveillance Strategy for Pancreatic Neuroendocrine Tumours: When and How to Do It?

Review article: Steroid-Refractory Acute Severe Ulcerative Colitis in Infliximab-Experienced Patients

Research article: Long-Term Follow-Up of Kidney Function after Acute Liver Failure or Acute Liver Injury: A Cohort Study

A Partner Publication by Karger

RESEARCH | OPEN ACCESS | Gastroenterology

GE – Portuguese Journal of Gastroenterology

(Continued on next page)

Karger

Printed in Portugal by Printipo: printipo@printipo.com

GE – Portuguese Journal of Gastroenterology

(Continued)

(Continued on next page)

GE – Portuguese Journal of Gastroenterology

Karger

www.karger.com
karger@karger.com

GE – Portuguese Journal of Gastroenterology

Journal Information

Guidelines for Authors
We strongly encourage authors to read the Guidelines for Authors at www.karger.com/pjg _guidelines prior to submitting an article

Journal Contact
For questions or comments, please contact the persons responsible who can be found at http://www.karger.com/Journal/Contact/272027

Aims and Scope

The *GE Portuguese Journal of Gastroenterology* (formerly *Jornal Português de Gastrenterologia*), founded in 1994, is the official publication of Sociedade Portuguesa de Gastrenterologia (Portuguese Society of Gastroenterology), Sociedade Portuguesa de Endoscopia Digestiva (Portuguese Society of Digestive Endoscopy) and Associação Portuguesa para o Estudo do Fígado (Portuguese Association for the Study of the Liver).
The journal publishes clinical and basic research articles on Gastroenterology, Digestive Endoscopy, Hepatology and related topics. Review articles, clinical case studies, images, letters to the editor and other articles such as recommendations or papers on gastroenterology clinical practice are also considered. Only articles written in English are accepted.

Price per printed issue: Free of charge
ERC-No.: 117866
Editor address: Rua Abranches Ferrão, nº 10–14º, PT–1600-001 Lisbon (Portugal)

ISSN Online Edition: 2387–1954

Journal Homepage: www.karger.com/pjg
Bibliographic Indices: This journal is regularly listed in bibliographic services, including PMC, PubMed, Web of Science, SciELO Citation Index, Google Scholar, DOAJ, Scopus, and WorldCat.

Publication Data: *GE Port J Gastroenterol* is published 6 times a year. Volume 31 with 6 issues appears in 2024.

Karger

© 2024 S. Karger AG, Basel
www.karger.com
karger@karger.com

GE – Portuguese Journal of Gastroenterology

Vol. 31, No. 5, 2024

Contents

Cover illustration
Anti-Reflux Mucosal Ablation: One More Kid in Town for the Treatment of Gastroesophageal Reflux Disease
From Garrido et. al., pp. 360–363

Appendiceal Submucosal Tumor: The Potential of Endoscopic Full-Thickness Resection in a Rare Entity
From Costa et. al., pp. 367–369

Karger

GE – Portuguese Journal of Gastroenterology

Karger

GE – Portuguese Journal of Gastroenterology

Letter to the Editor

GE Port J Gastroenterol 2024;31:303–305
DOI: 10.1159/000539161

Received: April 3, 2024
Accepted: April 25, 2024
Published online: June 3, 2024

All Neuroendocrine Tumors Seem to Look Alike but Some Look Alike More Than Others

Ana Paula Santos[a, b]

[a]Endocrinology Department, Portuguese Oncology Institute of Porto (IPO Porto), Porto Comprehensive Cancer Centre (P.CCC), Porto, Portugal; [b]Precancerous Lesions and Early Cancer Management Group (PRECAM), Research Center of IPO Porto (CI-IPOP), Portuguese Oncology Institute of Porto (IPO Porto), Porto Comprehensive Cancer Centre (P.CCC), Porto, Portugal

Keywords
Neuroendocrine tumors · Functioning neuroendocrine tumors · Hereditary neuroendocrine tumors · Multiple endocrine neoplasia

Todos os tumores neuroendócrinos parecem iguais, mas uns são mais iguais que outros

Palavras Chave
Tumores neuroendócrinos · TNE · TNE funcionante · TNE hereditário · Síndrome de neoplasia endócrina múltipla (MEN)

Digestive neuroendocrine tumors (DNETs) are still considered rare tumors, although their incidence has been rising since the 1970s [1, 2]. In the past 2 decades, important advances in diagnosis and treatment have been made, so since the 2010s, survival has increased as well [1, 2]. As a consequence, DNETs turned to be one of the most prevalent neoplasia (170,000 cases in the USA until 2020) [3]. Due to DNET heterogenicity and rarity, these tumors should be managed at reference centers by multidisciplinary teams including gastroenterology, endocrinology, pathology, surgery, medical oncology, interventional radiology, and nuclear medicine specialists, among others. According to recent guidance papers, small pancreatic neuroendocrine tumors (<2 cm) can be followed by "watch-wait" surveillance [4]. Nevertheless, some groups as the Portuguese Pancreatic Club, question this recommendation, arguing that beside the size, other preoperative factors may help stratify the risk of malignant behavior [5]. The question of functionality and hereditary should also be considered, as for instance, a small sporadic gastrinoma should be operated because of its metastatic potential [6]. Besides, although frequently benign, a small insulinoma <2 cm should also be treated because of life-threatening symptoms [6].

Unlike other cancers, DNET diagnostic and treatment goals are focused not only on tumor burden, but also on hormone secretion by the primary tumor and its metastasis. In contrast to the global rise in incidence, the proportion of functioning tumors has been decreasing, from 40 to 50% in older studies [7, 8], to the 15–30% actually described [9]. In recent studies, non-functioning pancreatic endocrine tumors were twice as frequent as functioning PETs [8]. Whenever this incidence proportion reduction is real or due to underdiagnosis of hypersecretion syndromes, or both is unknown, but the fact

karger@karger.com
www.karger.com/pjg

Correspondence to:
Ana Paula Santos, anapaulasantos@ipoporto.min-saude.pt

that these tumors are managed by several specialities and often not referred to experienced centers favours the consideration of the last hypothesis.

Duodenal and pancreatic gastrinomas can be easily missed since the spread use of proton pump inhibitors (PPIs) can mask traditional symptoms of peptic ulcer disease due to hypergastrinemia. Gastrinoma should be suspected in the presence of recurrent peptic ulcer disease, in the absence of *Helicobacter pylori*, in chronic diarrhea that responds to PPI, as well as in patients who do not tolerate PPI withdrawal due to severe dyspeptic symptoms [6, 10, 11]. According to 2023 ENETS recommendations, gastrinemia measurement is mandatory in all DP-NETs, when Zollinger-Ellison syndrome is suspected [6]. Hypoglycemia caused by insulinoma can present with neuroglycopenic symptoms such as confusion, blurred vision, and incoherent speech, besides the autonomic nervous system symptoms such as tremor, sweating, hunger, and tachycardia characteristic of hypoglycemia [6, 10, 11]. Additionally, during the course of the disease, progression and dedifferentiation of non-functioning metastatic tumors can be associated with de novo hypersecretion, sometimes with synchronous or metachronous secretion of multiple peptides and hormones [6, 10, 11]. Even metastatic midgut tumors associated with carcinoid syndrome only manifest the typical symptoms of flushing and diarrhea in advanced stages of the disease [11]. Symptoms mimicking irritable bowel syndrome are frequently found in early stages of the disease, when intermittent abdominal pain, nausea, vomiting, and acute changes of intestinal habits are often responsible for sporadic health care visits and are usually misdiagnosed as acute gastroenteritis or attributed to alimentary excesses [10, 11].

On the other side, hereditary syndromes should be suspected, particularly in patients diagnosed under 40 years old with DP-NETs, with two or more endocrine tumors or with a family history of endocrine tumors. Type 1 multiple endocrine neoplasia (MEN1), caused by inactivating mutations of menin gene is the most frequent hereditary syndrome associated with DP-NETs; however, other syndromes have recently been identified, as MEN4 caused by germline mutations of CDKN1B, encoding p27 protein. Von Hippel-Lindau disease; type 1 neurofibromatosis; and tuberous sclerosis complex should also be considered [6, 12].

In conclusion, although nonfunctioning DNETs are more frequent than functioning tumors, the possibility of hypersecretion must be kept in mind. Carcinoid syndrome must be excluded in all metastatic midgut NETs. DP-NETS should be carefully evaluated in order to avoid misdiagnosis of gastrinoma, insulinoma, and other rare functioning syndromes, as well as hereditary disease.

Acknowledgment

The author is grateful to Mariana P. Monteiro for revising and supporting the formatting of the manuscript.

Conflict of Interest Statement

The author declares no conflicts of interest.

Funding Sources

This work did not receive any funding either from public, commercial, or nonprofit agencies.

Author Contributions

A.P.S.: conceptualization and writing – original draft preparation.

References

1 Xu Z, Wang L, Dai S, Chen M, Li F, Sun J, et al. Epidemiologic trends of and factors associated with overall survival for patients with gastroenteropancreatic neuroendocrine tumors in the United States. JAMA Netw Open. 2021;4(9):e2124750. https://doi.org/10.1001/jamanetworkopen.2021.24750

2 White BE, Rous B, Chandrakumaran K, Wong K, Bouvier C, Van Hemelrijck M, et al. Incidence and survival of neuroendocrine neoplasia in England 1995–2018: a retrospective, population-based study. Lancet Reg Health Eur. 2022;23:100510. https://doi.org/10.1016/j.lanepe.2022.100510

3 Das S, Dasari A. Epidemiology, incidence, and prevalence of neuroendocrine neoplasms: are there global differences? Curr Oncol Rep. 2021;23(4):43–7. https://doi.org/10.1007/s11912-021-01029-7

4 Kos-Kudła B, Castaño JP, Denecke T, Grande E, Kjaer A, Koumarianou A, et al. European Neuroendocrine Tumour Society (ENETS) 2023 guidance paper for nonfunctioning pancreatic neuroendocrine tumours. J Neuroendocrinol. 2023;35(12):e13343. https://doi.org/10.1111/jne.13343

5 Bispo M, Marques S, Fernandes A, Rodrigues-Pinto E, Vilas-Boas F, Rio-Tinto R, et al. Portuguese pancreatic club perspective on the surveillance strategy for pancreatic neuroendocrine tumours: when and how to do it? GE Port J Gastroenterol. 2024:1–8. https://doi.org/10.1159/000535815

6 Grozinsky-Glasberg S, Davar J, Hofland J, Dobson R, Prasad V, Pascher A, et al. European neuroendocrine tumor society (ENETS) 2022 guidance paper for carcinoid syndrome and carcinoid heart disease. J Neuroendocrinol. 2022;34(7):e13146. https://doi.org/10.1111/jne.13146

7 Metz DC, Jensen RT. Gastrointestinal neuroendocrine tumors: pancreatic endocrine tumors. Gastroenterology. 2008; 135(5):1469–92. https://doi.org/10.1053/j.gastro.2008.05.047
8 Batukbhai BDO, De Jesus-Acosta A. The molecular and clinical landscape of pancreatic neuroendocrine tumors. Pancreas. 2019;48(1):9–21. https://doi.org/10.1097/mpa.0000000000001189
9 Magi L, Marasco M, Rinzivillo M, Faggiano A, Panzuto F. Management of functional pancreatic neuroendocrine neoplasms. Curr Treat Options Oncol. 2023;24(7):725–41. https://doi.org/10.1007/s11864-023-01085-0
10 Vinik AI, Gonzales MRC. New and emerging syndromes due to neuroendocrine tumors. Endocrinol Metab Clin North Am. 2011;40(1):19–63. https://doi.org/10.1016/j.ecl.2010.12.010
11 Hofland J, Kaltsas G, de Herder WW. Advances in the diagnosis and management of well-differentiated neuroendocrine neoplasms. Endocr Rev. 2020;41(2):371–403. https://doi.org/10.1210/endrev/bnz004
12 Oleinikov K, Grozinsky-Glasberg S. Hereditary syndromes associated with neuroendocrine tumors. Curr Opin Endocr Metab Res. 2021;18:230–5. https://doi.org/10.1016/j.coemr.2021.03.018

GE – Portuguese Journal of Gastroenterology

Review Article

GE Port J Gastroenterol 2024;31:306–313
DOI: 10.1159/000535815

Received: July 20, 2023
Accepted: November 24, 2023
Published online: January 16, 2024

Portuguese Pancreatic Club Perspective on the Surveillance Strategy for Pancreatic Neuroendocrine Tumours: When and How to Do It?

Miguel Bispo[a] Susana Marques[a] Alexandra Fernandes[b]
Eduardo Rodrigues-Pinto[c, d] Filipe Vilas-Boas[c, d] Ricardo Rio-Tinto[a]
Jacques Devière[a, e]
on behalf of the Portuguese Pancreatic Club, specialized section of the Portuguese Society of Gastroenterology

[a]Digestive Oncology Unit, Champalimaud Foundation, Lisbon, Portugal; [b]Department of Gastroenterology, Centro Hospitalar de Leiria, Leiria, Portugal; [c]Department of Gastroenterology, Centro Hospitalar Universitário de São João, Porto, Portugal; [d]Faculty of Medicine of the University of Porto, Porto, Portugal; [e]Department of Gastroenterology, Hepatopancreatology, and Digestive Oncology, Erasme University Hospital – Université Libre de Bruxelles, Brussels, Belgium

Keywords
Endoscopic ultrasound · Ki-67 proliferative index · Pancreatic neuroendocrine tumours · Surveillance

Abstract
Background: Pancreatic neuroendocrine tumours (pNETs) are a highly heterogeneous group of tumours with widely variable biological behaviour. The incidence of pNETs has risen exponentially over the last three decades, particularly for asymptomatic small pNETs (≤2 cm), due to the widespread use of cross-sectional imaging in clinical practice. ***Summary:*** Current consensus guidelines suggest that incidentally discovered pNETs ≤2 cm can be selectively followed due to the overall low risk of malignancy. Nevertheless, the "watch-and-wait" management strategy for small asymptomatic pNETs is still not widely accepted due to the lack of long-term data on the natural history of these small lesions. Additionally, it is clear that a subset of small pNETs may show malignant behaviour. ***Key Message:*** Given the non-negligible risk of malignancy even in small pNETs, it is of the utmost importance to identify other preoperative factors, other than size, that may help to stratify the risk of malignant behaviour and guide clinical management. In this article, the Portuguese Pancreatic Club reviews the importance of risk stratification of pNETs and presents an updated perspective on the surveillance strategy for sporadic well-differentiated pNETs.

Perspetiva do Clube Português de Pâncreas sobre a estratégia de vigilância dos tumores neuroendócrinos do pâncreas: quando e como vigiar?

Palavras Chave
Ecoendoscopia · Indice proliferativo Ki-67 · Tumores neuroendócrinos pancreáticos · pNETs · Vigilância

karger@karger.com
www.karger.com/pjg

Correspondence to:
Miguel Bispo, miguel.bispo@fundacaochampalimaud.pt

Resumo

Contexto: Os tumores neuroendócrinos do pâncreas (*pNETs*) correspondem a um grupo heterogéneo de tumores com comportamento biológico variável. A sua incidência aumentou exponencialmente nas últimas três décadas, particularmente à custa do diagnóstico incidental de *pNETs* de reduzidas dimensões (≤2 cm) devido à utilização crescente de exames de imagem seccional na prática clínica. ***Sumário:*** As normas de consenso internacionais sugerem que os *pNETs* ≤2 cm poderão ser seletivamente vigiados, dado o seu baixo risco global de comportamento maligno. No entanto, a estratégia proposta de "*watch and wait*" na abordagem dos *pNETs* assintomáticos ≤2cm não tem sido amplamente aceite devido à ausência de dados a longo-prazo relativos à sua história natural. Adicionalmente, é hoje evidente que um subgrupo destes pequenos tumores poderá apresentar comportamento maligno. ***Mensagens Chave:*** Dado o risco não desprezível de agressividade biológica mesmo nos *pNETs* incidentais de reduzidas dimensões, torna-se essencial identificar fatores pré-operatórios, para além da dimensão do tumor, que permitam estratificar o seu risco de malignidade e guiar a abordagem clínica. No presente artigo o Clube Português de Pâncreas apresenta uma perspectiva atual sobre a estratificação do risco e a estratégia a adoptar na vigilância dos *pNETs* esporádicos bem-diferenciados.

Introduction

Pancreatic neuroendocrine tumours (pNETs) comprise a heterogeneous group of neoplasms originating from the islets of Langerhans that exhibit distinct molecular and clinical features, with variable patterns of aggressiveness [1]. These tumours have been historically regarded as rare, but several lines of evidence support the hypothesis that the real prevalence of pNETs is much higher than that reported in population-based studies [1]. Large autopsy series have documented a pNET prevalence of 1.5–3%, mostly comprising small lesions [2, 3]. In a recent series of pancreatic surgical resection specimens for miscellaneous indications (other than pNETs), a prevalence of 4% of small incidental pNETs was reported by the pathologists [4]. The high prevalence of incidental pNETs documented in these studies supports the hypothesis that the risk of malignant behaviour is probably limited to a small fraction of cases and that most pNETs probably remain asymptomatic during lifetime [1, 4]. In fact, the incidence of pNETs has risen more than 6-fold over the last three decades, and this dramatic growth has been markedly greater for localized disease, possibly due to increased imaging diagnosis of asymptomatic, early-stage lesions [5, 6]. As diagnosis of pNETs become more frequent, it is of paramount importance to select which of these lesions will benefit from therapeutic intervention.

Clinically, pNETs are classified as functioning or non-functioning according to whether they secrete active hormones. In recent series, non-functioning pNETs represent up to 90% of all lesions [7]. Surgery is the standard of care for pNETs that cause symptoms of hormone secretion and for pNETs that are determined to pose a high risk of malignancy (including all pNETs >2 cm) or that have established malignant features depending on their clinicopathological features and stage [1]. However, the management of incidentally detected, non-functioning, smaller lesions (≤2 cm) remains controversial. In clinical practice, since the natural history of these small tumours is largely unknown, the management strategy depends essentially on the adequate weighting of the risks of overtreatment and undertreatment [8].

In this article, the Portuguese Pancreatic Club reviews the importance of risk stratification of pNETs and presents an updated perspective on the surveillance strategy for sporadic well-differentiated pNETs. A literature search was performed through May 2023, using PubMed, Embase and Cochrane library, with the search terms "pancreatic neuroendocrine tumour/neoplasm," "pNET/panNET/pNEN/panNEN," "endoscopic ultrasound," "Ki-67 proliferative index," "surveillance," and "follow-up." A cross-reference check was performed during full-text article review. Prospective studies, systematic reviews/meta-analyses and international consensus statements/management guidelines were preferred. The final manuscript was revised and approved by all the members of the Governing Board of the Portuguese Pancreatic Club.

Risk Stratification of pNETs: The Present and the Future

It is extremely difficult to predict the course of disease in a patient with a pNET. Most (>90%) pNETs in clinical practice are well-differentiated low-grade (G1, Ki-67 index <3%) or intermediate-grade (G2, Ki-67 index 3–20%) tumours and are associated with a relatively prolonged natural history, even when metastatic [9, 10].

One relevant point to consider is that, while histologic grade is a useful measure of prognosis (as a Ki-67 index >5% is linked to a higher risk of disease progression and postoperative recurrence), it is not an indicator of whether a pNET is benign or malignant [9, 10]. The only criteria for malignant behaviour are the presence of local invasion, metastases, or recurrent disease [10]. Taken together, disease stage (evaluated by imaging and classified according to the ENETS/AJCC classification [11, 12]) and tumour grade (based on histology/proliferation index and classified according to the WHO classification [13]) are the two major independent prognostic factors and should always be assessed in a patient with a pNET [14].

Earlier classification systems from the WHO incorporated tumour size (≤2 cm, >2 cm) into the staging criteria for sporadic pNETs [15]. There is evidence that larger tumours are more likely to be intermediate grade rather than low grade and that larger tumours are more often malignant and have somewhat poorer outcomes with a higher risk of disease recurrence [16]. However, size alone cannot determine the malignant potential of these lesions: tumours <2 cm can be malignant and tumours >2 cm can be benign [16]. In a recent multicenter retrospective cohort study of patients with non-functioning pNETs ≤2 cm who underwent surgery, one-fourth had at least one high-risk pathological factor (defined as Ki-67 > 3%, microvascular invasion, or positive nodal involvement, the latter present in 6% of the cases) [17]. These findings were similar to the results of a recent meta-analysis which showed that up to 20% of surgically resected small (≤2 cm) pNETs had malignant potential [18]. Given the non-negligible risk of malignant behaviour even in small pNETs, it is of the utmost importance to identify other preoperative factors, other than size, that may help to stratify the risk of malignancy.

Besides size >2 cm [16] and Ki-67 index >5% [9, 10, 19], some particular imaging features may predict a higher risk of malignancy. The presence of hypoenhanced/heterogeneous vascular pattern, as may be revealed by dynamic contrast-enhanced imaging techniques, has been linked to the presence of high-risk pathological features [17]. Importantly, microvessel density is inversely correlated to tumour grading in histologic samples, justifying the hypoenhanced/heterogeneous contrast pattern in dynamic studies in higher-grade tumours [17, 20]. The presence of calcifications on preoperative imaging has also been shown to be an independent predictive factor of lymph node metastasis in well-differentiated pNETs and tends to occur in larger and intermediate-grade tumours [21, 22]. Additionally, upstream dilatation of the main pancreatic duct (due to intraductal invasion) and other signs of invasive behaviour, such as dilatation of the common bile duct, irregular borders, or invasion of adjacent vessels, are highly suggestive of underlying malignancy [17, 23]. Conversely, cystic degeneration, which occurs in about 11–19% of all pNETs, is mostly found in low-grade pNETs (probably due to intratumoural bleeding) and has been linked to lower nodal invasion rate and to better prognosis in comparison to solid pNETs [23, 24]. Other series have documented similar survival outcomes and similar rates of lymph node metastasis between pNETs with and without a cystic component [25, 26].

The utility of currently available circulating markers such as chromogranin A as an aid in the diagnosis or follow-up of pNETs is limited. Regarding chromogranin A, sensitivity is very low in cases of localized disease or low metastatic burden, and false-positive results are common in several medical conditions, such as inflammatory diseases, renal failure, chronic atrophic gastritis and with the use of proton pump inhibitors [27]. The NETest is a novel RNA-based assay that has been shown to be superior to chromogranin A in multiple metrics. This novel test measures several circulating tumour transcripts and outperformed other pNET biomarkers for prediction of tumour burden, disease progression, and response to therapy in a recent prospective comparative study [28]. In recent years, various techniques of molecular biology (based on tumour tissue sampling and liquid biopsy) have shown promising results by identifying relevant factors for prognosis/risk stratification of pNETs. Moreover, the determination of the molecular basis of this heterogeneous disease will be crucial to the development of personalized therapies. Importantly, the presence of DAXX/ATRX loss has been shown to be an independent negative prognostic factor, and its determination in biopsies samples may be helpful in the decision-making process for pNETs ≤2 cm [29].

Role of Endoscopic Ultrasound: Guided Tissue Acquisition for Risk Stratification of pNETs

A preoperative histological diagnosis is of paramount importance for confirmation of the neuroendocrine nature of the pancreatic lesion, which needs to be differentiated from other hypervascular pancreatic lesions,

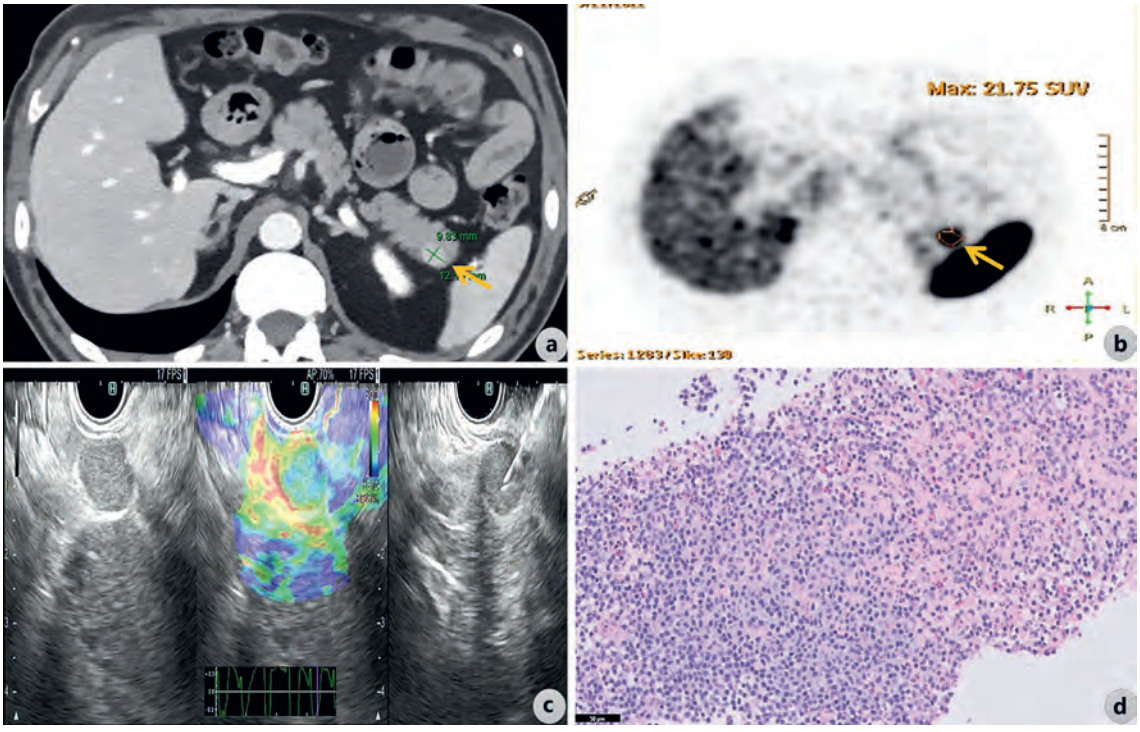


Fig. 1. A hypervascular nodule in the pancreatic tail suspected of being a pNET was documented on contrast-enhanced CT (arrow in **a**) and was positive for ^{68}Ga-labelled somatostatin analogues on ^{68}Ga-DOTA-NOC PET-CT, SUVmax 21.8 (arrow in **b**). Following EUS-guided FNB (**c** shows B-mode EUS, real-time elastography, and EUS-guided FNB), the final diagnosis of ectopic splenic tissue was made on pathology (**d**; H&E, scale bar corresponds to 50 μm).

such as solid-type serous cystic neoplasms, pancreatic lymphomas/plasmacytomas, hypervascular pancreatic metastases, or intrapancreatic accessory spleen lesions, some of which obviously not requiring surgical resection [30, 31]. Figure 1 shows a case involving a hypervascular nodule in the pancreatic tail that was suspected of being a pNET on computed tomography (CT) and on ^{68}Ga-DOTA-NOC PET-CT (positive for ^{68}Ga-labelled somatostatin analogues – SUVmax 21.8), with the final diagnosis of intrapancreatic accessory spleen following endoscopic ultrasound (EUS)-guided fine-needle biopsy (FNB). The 2020 European Society for Medical Oncology (ESMO) guidelines [14] recommend EUS as the optimal method for the diagnosis of small pNETs due to higher diagnostic sensitivity than cross-sectional imaging tests and because it allows for histologic diagnosis. For EUS-guided sampling of these lesions, ESMO recommends the use of a cutting FNB needle, in order to acquire a tissue core for immunohistochemistry [14]. The determination of the Ki-67 proliferation index in these samples allows assessment of tumour grade, which remains an important factor to consider in the choice between surgery and surveillance in small (≤2 cm) asymptomatic pNETs, together with other factors such as patient's age, performance status, tumour location and patient preference [14, 32]. In this regard, two recent studies have shown that the new end-cutting FNB needles outperform the traditional fine-needle aspiration (FNA) needles for Ki-67 index determination, demonstrating a closer match to surgical histology [33, 34]. This finding appears to be more significant in the assessment of small pNETs (≤2 cm), where EUS-FNA samples tend to underestimate the Ki-67 index, supporting that EUS-FNB should become the standard of care for grading small pNETs [33, 34]. There have been no prospective comparative studies evaluating different techniques of EUS-guided sampling in pNETs. Since pNETs are usually hypervascular, the non-suction/slow-pull technique has been suggested to reduce blood contamination of the specimen in a recent meta-analysis [35]. Additionally, the use of the fanning technique may be valuable for pNET sampling, particularly in large tumours that commonly

present intratumoural heterogeneity of Ki-67, with focal distribution of hotspots [32].

Novel molecular markers associated with a higher risk of metastasis (such as ARX-positivity, loss of DAXX/ATRX, and alternative lengthening of telomeres) may potentially be evaluated in EUS-FNB core samples [36, 37]. The European Neuroendocrine Tumour Society (ENETS) 2023 guideline highlights that the demonstration of DAXX/ATRX loss may favour surgical resection (as it is linked to a higher risk of malignant behaviour) and may be helpful in decision-making in ≤2 cm tumours [29].

How to Manage a Small (≤2 cm) Asymptomatic pNET

There is consensus among experts and guidelines that asymptomatic pNETs <1 cm can be safely followed, taking into account their indolent behaviour and extremely low metastatic potential [14, 29, 38–40]. Additionally, there is also agreement that well-differentiated pNETs >2 cm should be resected with curative intent (which should include regional lymphadenectomy) in surgically fit patients [14, 29, 38–40]. However, the management of asymptomatic pNETs between 1 and 2cm is still controversial [41]. The biological heterogeneity of these tumours poses challenges when choosing between surveillance and resection. Consensus recommendations addressing surveillance strategies are based on retrospective series with mid-term follow-up (generally <5 years) and on a limited number of systematic reviews of those studies [14, 29, 38–40]. While the 2020 ESMO guideline [14] endorses a "watchful waiting" approach for non-functioning pNETs <2 cm, current guidelines from the ENETS [29], the North American Neuroendocrine Tumor Society (NANETS) [40], and the National Comprehensive Cancer Network (NCCN) [39] recommend individualized management for pNETs between 1 and 2cm, based on the patient's age, comorbidities, extent of needed surgery, tumour grade, and patient preference. Other researchers, considering the metastatic potential of pNETs according to their size, have proposed 1.5 cm or 1.7 cm as triggers for surgical resection [42, 43]. Recently, a multicentre study reported that pNETs measuring 1.5–2cm had a much higher risk of lymph node metastasis than tumours <1.5 cm (17.9% vs. 8.7%), recommending surgical resection with lymphadenectomy for pNETs ≥1.5 cm [44].

Even though current guidelines recommend watchful waiting as a valid option for the management of small pNETs, a recent nationwide cancer analysis revealed that 70–80% of patients with small non-functioning pNETs have undergone resection [45]. This discrepancy between guideline recommendations and real-world data may come from the fact that there are no well-established features to accurately differentiate between low-risk and high-risk small pNETs [17]. The fear of disease progression should not be discounted, as some surgical cohorts report that 10–15% of small pNETs have malignant behaviour with regional or distant metastasis [46–48]. Our knowledge of the metastatic potential of small pNETs is based on studies that evaluated the pathological features of postsurgical specimens or studies that have compared survival between patients who have undergone upfront surgery versus those who were followed conservatively. Both study designs are associated with selection bias, and key findings have been mixed. Two systematic reviews comparing surveillance versus surgery in the management of asymptomatic small pNETs (≤2 cm) have shown that active surveillance seems to be safe at least with a mid-term follow-up [49, 50]. Recently, two prospective cohort studies, the ASPEN trial [51] and the PANDORA trial [52], have shown the safety and feasibility of active surveillance of small pNETs in the short-term, with a small fraction of patients (2%) undergoing surgery (mainly due to tumour growth) after a median follow-up of 2 years in the largest study [51]. However, to evaluate the oncological safety of watchful waiting in patients with small pNETs, longer follow-up is needed.

As there is clear evidence that a subset of small asymptomatic pNETs may demonstrate malignant behaviour (and that size is not a sufficient criterion for decision-making), additional features predictive of the biological behaviour of pNETs should be sought. Javed et al. [53] have recently proposed a predictive model for lymph node metastasis in small pNETs based on tumour grade and size. In this multicentre retrospective study, G2 grade (OR 3.51, 95% confidence interval 1.71–7.22) and tumour size (per mm increase, OR 1.14, 95% confidence interval 1.03–1.25) were strongly associated with nodal disease, and the authors developed a predictive model based on these two variables to identify distinct risk groups of nodal disease [53].

In conclusion, it appears reasonable that, in the rare instance of a small pNET that demonstrates worrisome features on imaging, including any sign of invasive behaviour, upfront surgery should be offered [14, 29, 40]. In the most common scenario of a pNET between 10 and 20 mm without worrisome features on imaging, EUS-FNB is a powerful tool to evaluate tumour grade (and eventually other markers, such as ATRX/DAXX loss), which must be considered in the decision-making process [29, 32–34]. The optimal Ki-67 index cut-off for stratifying pNETs into groups at high risk and low risk of malignant behaviour has been a matter of debate, with several studies pointing to a Ki-67 index cut-off of 5% as a threshold for surgery [19]. Importantly, the potential

benefit from surgery appears to be higher as the size of the tumour increases [16]. An incremental risk of nodal disease with increasing tumour size has been recently described as a continuous variable instead of a single cut-off for risk stratification [53]. Of course, the potential benefit from surgery is higher in younger patients with longer life expectancy and also whenever a less invasive surgical intervention may be feasible, as in pNETs located in the pancreatic body or tail [29, 40]. Finally, patient preference and access to long-term follow-up should also be carefully considered [40].

How to Do Surveillance

There are no prospective validation studies and no evidence-based guidelines regarding the optimal follow-up strategy [29, 38, 40]. Surveillance typically includes periodic cross-sectional imaging with CT or MRI. The NCCN states that MRI should be considered over CT to minimize radiation exposure [39]. According to the ENETS consensus guidelines [29, 38], small asymptomatic pNETs (≤2 cm) with a low Ki-67 index (≤5%) may be followed by MRI, EUS, or CT every 6–12 months, suggesting initial surveillance at shorter intervals during the first year and extending surveillance intervals up to 1 year in case of stability of imaging findings [38]. The recommended follow-up protocol in the ASPEN trial consisted of MRI or CT every 6 months for the first 2 years and yearly thereafter in the absence of significant changes on imaging [8]. A more intensive follow-up protocol, as proposed in the PANDORA trial [52], resulted in lower adherence by the physicians, who considered the follow-up intervals too short. In the watch-and-wait strategy, recommended criteria for surgery include tumour growth exceeding 5 mm/year, or up to a tumour size >2 cm, or the appearance of any worrisome features of invasive behaviour, such as main pancreatic duct dilatation, vascular involvement, or pathological lymph node enlargement [38, 52].

There is no established role for somatostatin analogue-based imaging (e.g., ^{68}Ga-DOTA-NOC PET-CT) or serum biomarkers in the follow-up of small pNETs, which should be used on a case-by-case basis at the physician's discretion [8, 29, 39, 40]. Somatostatin analogue-based imaging may be useful when there is a suspicion of tumour progression based on conventional imaging (CT or MRI), particularly to clarify the extent of disease [29, 40]. Although chromogranin A is commonly used during follow-up, its sensitivity and specificity are insufficient, and this serum marker rarely, if ever, influences management decisions [40]. The integration of novel biomarkers, such as the NETest, in the surveillance of pNETs still requires further study [40].

Key Points

- Disease stage (evaluated by imaging) and WHO tumour grade (based on the Ki-67 proliferation index, determined by histology) are the two major independent prognostic factors and should always be assessed in a patient with a newly diagnosed pNET.
- Besides size >2 cm and Ki-67 index >5%, several worrisome features on imaging (such as the presence of tumoural calcifications and upstream dilatation of the pancreatic duct) have been linked to a higher risk of disease progression, for which surgery is generally recommended.
- There is consensus that asymptomatic pNETs <1 cm can be safely followed.
- A subset of small asymptomatic pNETs between 1 and 2 cm may show malignant behaviour and additional features predictive of their biological behaviour should be sought.
- The role of EUS-FNB stands out particularly for the evaluation of small pNETs between 1 and 2 cm, allowing both histologic diagnosis, tumour grading, and, eventually, determination of ATRX/DAXX status.
- The new end-cutting FNB needles outperform the traditional FNA needles for Ki-67 index determination, demonstrating a closer match to surgical histology.
- An incremental risk of nodal disease with increasing tumour size has been recently described as a continuous variable instead of a single cut-off for risk stratification.
- The decision to follow a watch-and-wait strategy in a patient with an asymptomatic pNET between 1 and 2cm should be made on an individual case basis, after weighing risks and benefits.
- Criteria that should be considered in the decision-making process include the patient's life expectancy (age and comorbidities), imaging features, WHO tumour grade, extent of surgical resection required, and patient preference. Additional markers (potentially determined in EUS-FNB samples), such as DAXX/ATRX loss, may also be helpful.
- A proposed follow-up protocol for localized small pNETs consists of MRI or CT every 6 months for the first 2 years and yearly thereafter in the absence of significant changes on imaging. MRI should be considered over CT to minimize radiation exposure.

Statement of Ethics

Ethics Committee approval was not required due to the nature of the study.

Conflict of Interest Statement

All authors have no personal conflicts of interest or financial relationships relevant to this publication to disclose.

Author Contributions

Miguel Bispo: article concept and design, literature review, and draft of the manuscript. Susana Marques, Alexandra Fernandes, Eduardo Rodrigues-Pinto, Filipe Vilas-Boas, Ricardo Rio-Tinto, and Jacques Devière: literature review and critical review of the manuscript.

Funding Sources

No funding was received.

Data Availability Statement

All data analysed during this study are included in the article. Further enquiries can be directed to the corresponding author.

References

1 Andreasi V, Partelli S, Muffatti F, Manzoni MF, Capurso G, Falconi M. Update on gastroenteropancreatic neuroendocrine tumors. Dig Liver Dis. 2021;53(2):171–82.
2 Kimura W, Kuroda A, Morioka Y. Clinical pathology of endocrine tumors of the pancreas. Analysis of autopsy cases. Dig Dis Sci. 1991;36(7):933–42.
3 Kishi S, Sakamoto K, Mori M, Isogawa A, Shiba T. Asymptomatic insulinoma: a case report and autopsy series. Diabetes Res Clin Pract. 2012;98(3):445–51.
4 Partelli S, Giannone F, Schiavo Lena M, Muffatti F, Andreasi V, Crippa S, et al. Is the real prevalence of pancreatic neuroendocrine tumors underestimated? A retrospective study on a large series of pancreatic specimens. Neuroendocrinology. 2019;109(2):165–70.
5 Dasari A, Shen C, Halperin D, Zhao B, Zhou S, Xu Y, et al. Trends in the incidence, prevalence, and survival outcomes in patients with neuroendocrine tumors in the United States. JAMA Oncol. 2017;3(10):1335–42.
6 Kuo EJ, Salem RR. Population-level analysis of pancreatic neuroendocrine tumors 2 cm or less in size. Ann Surg Oncol. 2013;20(9):2815–21.
7 Gorelik M, Ahmad M, Grossman D, Grossman M, Cooperman AM. Nonfunctioning incidental pancreatic neuroendocrine tumors: who, when, and how to treat? Surg Clin North Am. 2018;98(1):157–67.
8 Partelli S, Ramage JK, Massironi S, Zerbi A, Kim HB, Niccoli P, et al. Management of Asymptomatic Sporadic Nonfunctioning Pancreatic Neuroendocrine Neoplasms (ASPEN) ≤2 cm: study protocol for a prospective observational study. Front Med. 2020;7:598438.
9 Boninsegna L, Panzuto F, Partelli S, Capelli P, Delle Fave G, Bettini R, et al. Malignant pancreatic neuroendocrine tumour: lymph node ratio and Ki67 are predictors of recurrence after curative resections. Eur J Cancer. 2012;48(11):1608–15.
10 Panzuto F, Boninsegna L, Fazio N, Campana D, Pia Brizzi M, Capurso G, et al. Metastatic and locally advanced pancreatic endocrine carcinomas: analysis of factors associated with disease progression. J Clin Oncol. 2011;29(17):2372–7.
11 Perren A, Couvelard A, Scoazec JY, Costa F, Borbath I, Delle Fave G, et al. ENETS consensus guidelines for the standards of care in neuroendocrine tumors: pathology: diagnosis and prognostic stratification. Neuroendocrinology. 2017;105(3):196–200.
12 O'Sullivan B, Brierley J, Byrd D, Bosman F, Kehoe S, Kossary C, et al. The TNM classification of malignant tumours-towards common understanding and reasonable expectations. Lancet Oncol. 2017;18(7):849–51.
13 Rindi G, Mete O, Uccella S, Basturk O, La Rosa S, Brosens LAA, et al. Overview of the 2022 WHO classification of neuroendocrine neoplasms. Endocr Pathol. 2022;33(1):115–54.
14 Pavel M, Öberg K, Falconi M, Krenning EP, Sundin A, Perren A, et al. Gastroenteropancreatic neuroendocrine neoplasms: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2020;31(7):844–60.
15 Klöppel G. Classification and pathology of gastroenteropancreatic neuroendocrine neoplasms. Endocr Relat Cancer. 2011;18(Suppl 1):S1–16.
16 Bettini R, Partelli S, Boninsegna L, Capelli P, Crippa S, Pederzoli P, et al. Tumor size correlates with malignancy in nonfunctioning pancreatic endocrine tumor. Surgery. 2011;150(1):75–82.
17 Nanno Y, Toyama H, Matsumoto I, Uemura J, Asari S, Goto T, et al. Reappraisal of malignant risk assessment for small (≤20 mm) non-functioning pancreatic neuroendocrine tumors. Ann Surg Oncol. 2023;30(6):3493–500.
18 Ricci C, Casadei R, Taffurelli G, Pacilio CA, Campana D, Ambrosini V, et al. Sporadic small (≤20 mm) nonfunctioning pancreatic neuroendocrine neoplasm: is the risk of malignancy negligible when adopting a more conservative strategy? A systematic review and meta-analysis. Ann Surg Oncol. 2017;24(9):2603–10.
19 Genç CG, Falconi M, Partelli S, Muffatti F, van Eeden S, Doglioni C, et al. Recurrence of pancreatic neuroendocrine tumors and survival predicted by Ki67. Ann Surg Oncol. 2018;25(8):2467–74.
20 Ishikawa T, Itoh A, Kawashima H, Ohno E, Matsubara H, Itoh Y, et al. Usefulness of EUS combined with contrast-enhancement in the differential diagnosis of malignant versus benign and preoperative localization of pancreatic endocrine tumors. Gastrointest Endosc. 2010;71(6):951–9.
21 Poultsides GA, Huang LC, Chen Y, Visser BC, Pai RK, Jeffrey RB, et al. Pancreatic neuroendocrine tumors: radiographic calcifications correlate with grade and metastasis. Ann Surg Oncol. 2012;19(7):2295–303.
22 Makris EA, Cannon JGD, Norton JA, Lopez-Aguiar AG, Dillhoff M, Beal E, et al. Calcifications and cystic morphology on preoperative imaging predict survival after resection of pancreatic neuroendocrine tumors. Ann Surg Oncol. 2023;30(4):2424–30.
23 Attili F, Capurso G, Vanella G, Fuccio L, Delle Fave G, Costamagna G, et al. Diagnostic and therapeutic role of endoscopy in gastroenteropancreatic neuroendocrine neoplasms. Dig Liver Dis. 2014;46(1):9–17.
24 Khalil A, Ewald J, Marchese U, Autret A, Garnier J, Niccoli P, et al. A single-center experience with pancreatic cystic neuroendocrine tumors. World J Surg Oncol. 2020;18(1):208.
25 Nakashima Y, Ohtsuka T, Nakamura S, Mori Y, Nakata K, Miyasaka Y, et al. Clinicopathological characteristics of non-functioning cystic pancreatic neuroendocrine tumors. Pancreatology. 2019;19(1):50–6.
26 Gaujoux S, Tang L, Klimstra D, Gonen M, Brennan MF, D'Angelica M, et al. The outcome of resected cystic pancreatic endocrine neoplasms: a case-matched analysis. Surgery. 2012;151(4):518–25.
27 Marotta V, Zatelli MC, Sciammarella C, Ambrosio MR, Bondanelli M, Colao A, et al. Chromogranin A as circulating marker for diagnosis and management of neuroendocrine neoplasms: more flaws than fame. Endocr Relat Cancer. 2018;25(1):R11–R29.

28 Modlin IM, Kidd M, Falconi M, Filosso PL, Frilling A, Malczewska A, et al. A multigenomic liquid biopsy biomarker for neuroendocrine tumor disease outperforms CgA and has surgical and clinical utility. Ann Oncol. 2021;32(11):1425–33.
29 Kos-Kudła B, Castaño JP, Denecke T, Grande E, Kjaer A, Koumarianou A, et al. European Neuroendocrine Tumour Society (ENETS) 2023 guidance paper for nonfunctioning pancreatic neuroendocrine tumours. J Neuroendocrinol. 2023;35(12):e13343.
30 Ishii T, Katanuma A, Toyonaga H, Chikugo K, Nasuno H, Kin T, et al. Role of endoscopic ultrasound in the diagnosis of pancreatic neuroendocrine neoplasms. Diagnostics. 2021;11(2):316.
31 Ardengh JC, Lopes CV, Kemp R, Lima-Filho ER, Venco F, Santos JS. Pancreatic splenosis mimicking neuroendocrine tumors: microhistological diagnosis by endoscopic ultrasound guided fine needle aspiration. Arq Gastroenterol. 2013;50(1):10–4.
32 Tacelli M, Bina N, Crinò SF, Facciorusso A, Celsa C, Vanni AS, et al. Reliability of grading preoperative pancreatic neuroendocrine tumors on EUS specimens: a systematic review with meta-analysis of aggregate and individual data. Gastrointest Endosc. 2022;96(6):898–908.e23.
33 Leeds JS, Nayar MK, Bekkali NLH, Wilson CH, Johnson SJ, Haugk B, et al. Endoscopic ultrasound-guided fine-needle biopsy is superior to fine-needle aspiration in assessing pancreatic neuroendocrine tumors. Endosc Int Open. 2019;7(10):E1281–7.
34 Crinò SF, Ammendola S, Meneghetti A, Bernardoni L, Conti Bellocchi MC, Gabbrielli A, et al. Comparison between EUS-guided fine-needle aspiration cytology and EUS-guided fine-needle biopsy histology for the evaluation of pancreatic neuroendocrine tumors. Pancreatology. 2021;21(2):443–50.
35 Capurso G, Archibugi L, Petrone MC, Arcidiacono PG. Slow-pull compared to suction technique for EUS-guided sampling of pancreatic solid lesions: a meta-analysis of randomized controlled trials. Endosc Int Open. 2020;8(5):E636–43.
36 Melita G, Pallio S, Tortora A, Crinò SF, Macrì A, Dionigi G. Diagnostic and interventional role of endoscopic ultrasonography for the management of pancreatic neuroendocrine neoplasms. J Clin Med. 2021;10(12):2638.
37 Arakelyan J, Zohrabyan D, Philip PA. Molecular profile of pancreatic neuroendocrine neoplasms (PanNENs): opportunities for personalized therapies. Cancer. 2021;127(3):345–53.
38 Falconi M, Eriksson B, Kaltsas G, Bartsch DK, Capdevila J, Caplin M, et al. ENETS consensus guidelines update for the management of patients with functional pancreatic neuroendocrine tumors and non-functional pancreatic neuroendocrine tumors. Neuroendocrinology. 2016;103(2):153–71.
39 National Comprehensive Cancer Network (NCCN). NCCN clinical pratice guidelines in oncolology. Available from: https://www.nccn.org/professionals/physician_gls (accessed on June 04, 2023).
40 Howe JR, Merchant NB, Conrad C, Keutgen XM, Hallet J, Drebin JA, et al. The North American neuroendocrine tumor society consensus paper on the surgical management of pancreatic neuroendocrine tumors. Pancreas. 2020;49(1):1–33.
41 Mansour JC, Chavin K, Morris-Stiff G, Warner SG, Cardona K, Fong ZV, et al. Management of asymptomatic, well-differentiated PNETs: results of the Delphi consensus process of the Americas Hepato-Pancreato-Biliary Association. HPB. 2019;21(5):515–23.
42 Kishi Y, Shimada K, Nara S, Esaki M, Hiraoka N, Kosuge T. Basing treatment strategy for non-functional pancreatic neuroendocrine tumors on tumor size. Ann Surg Oncol. 2014;21(9):2882–8.
43 Regenet N, Carrere N, Boulanger G, de Calan L, Humeau M, Arnault V, et al. Is the 2-cm size cutoff relevant for small nonfunctioning pancreatic neuroendocrine tumors: a French multicenter study. Surgery. 2016;159(3):901–7.
44 Dong DH, Zhang XF, Poultsides G, Rocha F, Weber S, Fields R, et al. Impact of tumor size and nodal status on recurrence of nonfunctional pancreatic neuroendocrine tumors ≤2 cm after curative resection: a multi-institutional study of 392 cases. J Surg Oncol. 2019;120(7):1071–9.
45 Mintziras I, Keck T, Werner J, Fichtner-Feigl S, Wittel U, Senninger N, et al. Implementation of current ENETS guidelines for surgery of small (≤2 cm) pancreatic neuroendocrine neoplasms in the German surgical community: an analysis of the prospective DGAV StuDoQ|Pancreas registry. World J Surg. 2019;43(1):175–82.
46 Vega EA, Kutlu OC, Alarcon SV, Salehi O, Kazakova V, Kozyreva O, et al. Clinical prognosticators of metastatic potential in patients with small pancreatic neuroendocrine tumors. J Gastrointest Surg. 2021;25(10):2593–9.
47 Sharpe SM, In H, Winchester DJ, Talamonti MS, Baker MS. Surgical resection provides an overall survival benefit for patients with small pancreatic neuroendocrine tumors. J Gastrointest Surg. 2015;19(1):117–23; discussion 123.
48 Gratian L, Pura J, Dinan M, Roman S, Reed S, Sosa JA. Impact of extent of surgery on survival in patients with small nonfunctional pancreatic neuroendocrine tumors in the United States. Ann Surg Oncol. 2014;21(11):3515–21.
49 Partelli S, Cirocchi R, Crippa S, Cardinali L, Fendrich V, Bartsch DK, et al. Systematic review of active surveillance versus surgical management of asymptomatic small nonfunctioning pancreatic neuroendocrine neoplasms. Br J Surg. 2017;104(1):34–41.
50 Sallinen V, Le Large TY, Galeev S, Kovalenko Z, Tieftrunk E, Araujo R, et al. Surveillance strategy for small asymptomatic nonfunctional pancreatic neuroendocrine tumors - a systematic review and meta-analysis. HPB. 2017;19(4):310–20.
51 Partelli S, Massironi S, Zerbi A, Niccoli P, Kwon W, Landoni L, et al. Management of asymptomatic sporadic non-functioning pancreatic neuroendocrine neoplasms no larger than 2 cm: interim analysis of prospective ASPEN trial. Br J Surg. 2022;109(12):1186–90.
52 Heidsma CM, Engelsman AF, van Dieren S, Stommel MWJ, de Hingh I, Vriens M, et al. Watchful waiting for small non-functional pancreatic neuroendocrine tumours: nationwide prospective cohort study (PANDORA). Br J Surg. 2021;108(8):888–91.
53 Javed AA, Pulvirenti A, Zheng J, Michelakos T, Sekigami Y, Razi S, et al. A novel tool to predict nodal metastasis in small pancreatic neuroendocrine tumors: a multicenter study. Surgery. 2022;172(6):1800–6.

GE – Portuguese Journal of Gastroenterology

Review Article

GE Port J Gastroenterol 2024;31:314–324
DOI: 10.1159/000537693

Received: November 27, 2023
Accepted: January 31, 2024
Published online: March 21, 2024

Steroid-Refractory Acute Severe Ulcerative Colitis in Infliximab-Experienced Patients

Joana Revés[a] Ana Catarina Bravo[a] Catarina Neto Nascimento[a] Bárbara Morão[a] Catarina Frias-Gomes[a] Lídia Roque Ramos[a] Luísa Glória[a] Joana Torres[a, b] Carolina Palmela[a]

[a]Gastroenterology Department, Hospital Beatriz Ângelo, Loures, Portugal; [b]Faculty of Medicine, University of Lisbon, Lisbon, Portugal

Keywords
Acute severe ulcerative colitis · Infliximab · Janus kinase inhibitors · Cyclosporine

Abstract
Acute severe ulcerative colitis (ASUC) is a potentially life-threatening complication of ulcerative colitis (UC) that can lead to significant morbidity and mortality, with a substantial number of patients needing colectomy. Infliximab (IFX) has been increasingly used as a rescue therapy for patients who have failed intravenous steroids and has been more frequently used as an induction and maintenance therapy in moderate-to-severe UC. Therefore, the number of patients admitted with ASUC previously exposed to IFX has been increasing, raising additional challenges in the medical management of these patients to avoid emergent colectomy. This narrative review intends to summarise the most recent evidence in the medical management of steroid-refractory ASUC patients previously exposed to IFX and to propose a treatment algorithm for approaching this difficult-to-treat group of patients.

Tratamento da colite ulcerosa aguda grave refratária aos corticoides em doentes previamente expostos a infliximab

Palavras Chave
Colite ulcerosa aguda grave · Infliximab · Inibidores JAK · Ciclosporina

Resumo
A colite ulcerosa aguda grave (CUAG) é uma complicação potencialmente fatal da colite ulcerosa (CU), que pode levar a significativa morbilidade e mortalidade, com um número substancial de doentes a necessitar de colectomia. O uso de infliximab (IFX) como terapêutica de resgate em doentes sem resposta a corticoterapia endovenosa tem vindo a aumentar, bem como a sua utilização como terapêutica de indução e manutenção em doentes com CU moderada-grave. Assim, o número de doentes hospitalizados com CUAG que já estiveram previamente expostos ao IFX tem vindo a aumentar, levantando novos desafios na abordagem médica destes

karger@karger.com
www.karger.com/pjg

Correspondence to:
Joana Revés, joanareves94@gmail.com

doentes, de forma a evitar a colectomia emergente. Esta revisão narrativa tem como objetivo sumarizar a evidência mais recente na abordagem médica da CUAG refratária aos corticoides em doentes previamente expostos ao IFX e propor um algoritmo terapêutico para abordar este grupo desafiante de doentes.

Introduction

Acute severe ulcerative colitis (ASUC) is a potentially life-threatening complication of ulcerative colitis (UC), which can occur at least once during the disease course in 25% of the patients. Moreover, ASUC can be the presenting feature in 10–20% of the patients with UC [1]. Mortality in ASUC has been decreasing in recent years due to the use of intravenous steroids and early colectomy for non-responders, but remains at approximately 1%, being as high as 4% for older patients [2]. Despite improvements in mortality, ASUC is still associated with significant morbidity and approximately 30% of the patients need colectomy and temporary or definitive ileostomy [3, 4]. Even though there was a 4%/year reduction in short-term colectomy rates after admission for ASUC, long-term and emergency colectomy rates remain unchanged [5]. Therefore, early identification, accurate risk stratification, and immediate, appropriate, and intensive management are needed to minimise morbidity, colectomy, and mortality.

Infliximab (IFX) and cyclosporine (CyA) represent the sole-approved drugs for rescue medical therapy in patients with ASUC when standard steroid treatment proves ineffective. Given its ease of use and the concerns with CyA short-term toxicity, IFX has become a common first-line salvage therapy in this setting in many countries. Nevertheless, IFX has the limitation of being associated with 20–30% primary non-response. Furthermore, given the recommendations to use anti-tumour necrosis factor (TNF) as a first-line therapy in moderate-severe UC in many countries, such as Portugal, and the tendency for earlier use of advanced therapy in outpatients with moderate-severe UC, there is an increasing number of patients being admitted for ASUC, who have already failed or lost response to IFX [6]. Therefore, in an episode of steroid-refractory ASUC in this subset of patients, IFX ceases to be an appropriate rescue medical therapy and different salvage therapies to circumvent the need for colectomy may be needed. However, there is a paucity of data in the literature on how to approach these difficult-to-treat group of patients. This narrative review intends to provide a comprehensive summary of the most recent data on this subject, particularly on the role of CyA in patients previously exposed to IFX, new maintenance therapies after CyA induction, and new emerging drugs for ASUC.

Standard Salvage Medical Therapy

ASUC is clinically defined by the Truelove and Witts criteria, and these patients have indication to start intravenous steroids [7]. Response is assessed after 3 days, as proposed by the Oxford criteria. Non-responders (≥8 stools/day or 3–8 stools/day and CRP >45 mg/L) face an 85% colectomy risk. If steroids fail, patients can either be submitted to colectomy or escalate to a second-line medical therapy, called salvage or rescue therapy.

CyA, a calcineurin inhibitor that selectively inhibits T-cell immunity, and IFX, a monoclonal antibody against the TNFα, are established salvage therapies for steroid-refractory patients with ASUC. CyA was the first therapy to be approved, with the original randomized, double-blinded, placebo-controlled trial published in 1994 and demonstrating an 82% improvement within 7 days on a dose of 4 mg/kg compared to a 0% response in the placebo group ($p < 0.01$) [8]. As CyA side effects seemed to be mainly dose-dependent, a further study demonstrated that a lower dose of CyA (2 mg/kg) had equivalent efficacy [9]. IFX was later approved for use in this context with a significantly lower rate of colectomy within 3 months of therapy when compared with placebo (OR 4.9, 95% CI 1.4–17, $p = 0.017$) and without significant side effects [10].

The CYSIF trial, conducted by the GETAID, was the first head-to-head, randomized controlled trial (RCT) comparing the efficacy and safety of both CyA and IFX. The study included 115 patients, and none had previously been exposed to any of the drugs. CyA was administered initially through continuous intravenous infusion at 2 mg/kg/day and transitioned to oral formulation at 4 mg/kg/day in divided doses for 98 days, adjusted according to serum concentrations. IFX was administered with an initial 5 mg/kg infusion and additional infusions on days 14 and 42 for responders. Both groups started azathioprine after 1 week. The trial, designed as a superiority trial, revealed no significant differences (60% for CyA, 54% for IFX, absolute risk difference 6%, 95% CI –7 to 19, $p = 0.52$) on the primary outcome, which was a composite outcome for treatment failure (absence of clinical response or steroid-free remission during follow-up or an adverse event leading to treatment interruption,

colectomy, or death). There was also no difference in colectomy-free survival at 3 months of follow-up [11].

Subsequent head-to-head trials, such as the CONSTRUCT trial, included 270 participants who could not have been exposed to either IFX or CyA in the 3 months before admission. IFX was given at a dose of 5 mg/kg at weeks 0, 2, and 6 and CyA at a dose of 2 mg/kg/day by continuous infusion for up to 7 days, followed by oral CyA at 5.5 mg/kg/day for 12 weeks. After this, therapy was at the discretion of the medical team. The primary outcome was quality-adjusted survival evaluated sequentially until 3 years of follow-up, and there were no differences between groups. There were also no differences in the in-hospital and overall colectomy rates [12].

Although these studies demonstrated no differences between CyA and IFX as salvage therapies for steroid-refractory ASUC patients, it is worth mentioning that in all these studies, IFX was used on a regular scheme and no dose optimization was carried. Severe bowel inflammation seems to be associated with increased faecal loss of IFX as was highlighted in the study by Brandse et al. [13] where it was demonstrated that patients that were clinical non-responders at week 2 had significantly higher faecal concentrations of IFX after the first day of treatment than patients that were clinical responders ($p = 0.0047$). Moreover, another study demonstrated that patients with a higher baseline C-reactive protein had significantly lower serum concentrations of the drug and that patients with low serum albumin and higher baseline faecal calprotectin levels also had a trend towards lower serum IFX concentrations [14]. This raised the possibility that an accelerated IFX regimen could be more effective than a standard induction regimen, although most studies to date have had negative results as was demonstrated by a systematic review where there were no differences in colectomy rates between both accelerated and standard IFX groups [15]. More recently, a retrospective study with a propensity score-matched cohort of steroid-refractory ASUC patients demonstrated that an accelerated induction regimen of IFX seems to be associated with lower short-term colectomy rates but not long-term colectomy rates [16]. The differences in these results can be partially explained by inadequate statistical power due to limited sample sizes and by a significant variability in clinical practice patterns in the management of hospitalized UC patients as was shown by a survey study among experienced IBD centres [17]. Results from the PREDICT-UC (NCT02770040) which is an open-label multi-centre RCT to assess whether an accelerated (5 mg/kg at weeks 0, 1 and 3) or intensified (10 mg/kg at weeks 0 and 1) IFX induction regimen is superior to standard induction in ASUC will help clarify this question.

Despite differences in induction regimens that need to be clarified to better compare the efficacy of both drugs, these studies have also demonstrated high rates of colectomy in the long term, low prevalence of sustained remission, and need to switch therapies, suggesting that it is also needed to better understand maintenance regimens which may have a significant contribution to the long-term efficacy of both drugs [18, 19]. In a systematic review and meta-analysis, Narula et al. demonstrated through the analysis of non-RCTs that IFX was associated with a lower 12-month colectomy rate compared to CyA, although no differences were found in the 3-month colectomy rate [20]. One could hypothesize that this could be related to a longer persistence of IFX as it is used as induction and maintenance therapy as opposed to CyA which is only used for induction, with patients usually transitioned to azathioprine in the past. In the CONSTRUCT trial, after 12 weeks of follow-up, all treatments were at the discretion of the physician and only less than half of the patients were started on immunosuppressants in both groups [12]. Also, in the long-term follow-up of the patients in the CYSIF trial, a higher proportion of patients initially treated with CyA required subsequent systemic therapies when compared with those who received IFX at inclusion, with nearly half of patients first treated with CyA needing to switch quickly to IFX, within 1 year. After a median follow-up of 5 years, no differences were found between colectomy-free survival rates (61.5% vs. 65.1% for CyA and IFX, respectively, $p = 0.97$), although they were relatively higher when compared with the CONSTRUCT trial where the maintenance treatment was left at the discretion of the practising physician [12, 19]. Apart from efficacy, most studies have demonstrated no differences in terms of safety between IFX and CyA, although CyA is known to be associated with minor side effects (hypokalaemia, hypocalcaemia, tremors, paraesthesia, malaise, headache, abnormal liver function tests, gingival hyperplasia, and hirsutism) and with some major complications that despite being uncommon can be severe (hypertension, nephrotoxicity, opportunistic infections, and neurotoxicity) [21].

Salvage Therapy for IFX-Experienced Patients

CyA as a Salvage Therapy Followed by Non-Anti-TNF Biologics for Maintenance

In many countries, IFX has been chosen as the first-line salvage therapy for patients with steroid-resistant ASUC due to its ease of use, potential for transitioning to maintenance therapy, and extensive experience with the drug. However, CyA represents a valuable and

cost-effective alternative, especially for patients with contraindications to anti-TNF therapy or those who have previously failed IFX. Limited studies directly address the use of CyA in ASUC patients previously exposed to IFX, with most evidence focusing on sequential rescue therapy with both drugs in the same ASUC episode.

In one study of 40 patients on sequential rescue therapy, the colectomy-free survival rate was 65% at 1 month and 42% at 1 year, despite 40% of patients experiencing adverse events that did not worthy discontinuation of the drug [22]. Another recent review of 81 patients demonstrated a colectomy-free rate of 42% [23]. While these results seem promising for avoiding colectomy in a specific group of patients, the overall colectomy rate remains high, and there are significant adverse events. Caution is advised when considering this strategy, given the limited and heterogeneous evidence based on small patient numbers.

However, patients requiring sequential rescue therapy with either CyA or IFX during the same episode of ASUC likely represent a more challenging group with a more severe form of the disease and a higher risk of adverse events. This differs from patients who previously experienced a loss of response to IFX and then developed ASUC. It is plausible that using CyA in this context could yield more positive outcomes, but additional data are necessary to confirm this, particularly regarding safety.

It is important to note that CyA should only be used when serum level measurements are available and as a bridge to other maintenance therapy. Azathioprine is the most well-established maintenance therapy in these cases. However, in the long term, azathioprine is poorly tolerated in many patients, it can be associated with an increased risk of malignancy (particularly in older patients and without previous exposure to the Epstein-Barr virus), and in patients who have been previously exposed to IFX, it may be insufficient as a maintenance therapy. Therefore, in patients with steroid-refractory ASUC who have previously failed IFX and who are now rescued with CyA, other maintenance therapies are needed.

Data on the use of CyA followed by maintenance with vedolizumab or ustekinumab are scarce and mostly retrospective. In three studies with variable sample sizes, with different CyA induction regimens and with different timings of initiation of vedolizumab, the use of CyA for induction followed by maintenance with vedolizumab allowed more than two-thirds of the patients to avoid colectomy at 1 year of therapy without significant safety issues [24–26]. In a small prospective trial of 17 patients who received induction therapy with CyA and were maintained with vedolizumab, the colectomy-free survival at 1 year was 82% and 71% were in endoscopic remission by week 52 (shown in Table 1). Despite the heterogeneity in study groups, in a recent review, where only those patients fulfilling the ASUC criteria were considered, the colectomy-free rate was still high (71%) [23]. Regarding the bridging to ustekinumab, the evidence is even more scarce, being mostly of retrospective nature with low sample sizes (shown in Table 2). However, the results seem promising with none of the patients needing colectomy during follow-up [27–29].

Therefore, both vedolizumab and ustekinumab may become future maintenance therapies following induction with CyA in ASUC, with a more favourable safety profile when compared with thiopurines and being a reasonable option when patients have already failed anti-TNFs. Further prospective RCTs are needed to assess which therapy should be preferred, and what is the best timing to initiate the bridging following induction and to evaluate the long-term outcomes of these strategies.

Tacrolimus as an Alternative Calcineurin Inhibitor

Tacrolimus (TC) is a calcineurin inhibitor with a more potent inhibitory effect on activated T cells compared with CyA and with good bioavailability even when administered orally that can be used as an alternative therapy in patients with severe steroid-refractory UC. The first randomized study to elucidate the role of TC as an oral therapy for hospitalized patients with ASUC was published in 2006 and demonstrated that there was a dose-dependent efficacy and safety of oral TC with an optimal treatment target between 10 and 15 ng/mL [30]. In 2016, a systematic review with meta-analysis including 2 RCTs and 23 observational studies demonstrated that in severe and steroid-refractory UC patients, TC was associated with short-term high-clinical response and with colectomy-free rates that were as high as 70% after 12 months of follow-up, without increased risk of severe adverse events [31]. Also, a systematic review and network meta-analysis combined with benefit-risk analysis to simultaneously compare the efficacy (clinical response and colectomy-free rate) and safety of different therapies in severe steroid-refractory UC found that IFX was the most effective therapy, followed by CyA, TC, and placebo, though the differences between the three agents seemed small, suggesting also a role for TC in this setting. However, these results need to be interpreted with caution as there is no mean to statistically assess the difference between the benefits-risk of each therapy [32]. Also, most studies focus on outpatients with moderate-to-severe UC and, thus, there are insufficient data on the role of TC in ASUC patients. Moreover, most of the

Table 1. CyA plus vedolizumab as rescue therapy in steroid-refractory ASUC

Study	Sample size	CyA induction regimen	Vedolizumab maintenance regimen	Colectomy during follow-up	SAE
Pellet et al. [26], Clin Gastroenterol Hepatol, 2019	39 (36 [92%] previously exposed to anti-TNF)	Continuous IV infusion 2 mg/kg/day or orally at 4 mg/kg/day (serum concentration 150 and 250 ng/mL), followed by oral CyA in IV responders given twice a day at 4 mg/kg/day during 3 months	300 mg at weeks 0, 2, and 6, and then every 8 weeks. Could be prescribed every 4 weeks from week 10 (67% had dose escalation – 19 patients at week 10 and 7 patients after week 14). Vedolizumab was started after a median interval of 7 days (range 0–180) after CyA (depending on wait for approval)	11/39 (28%)	4/39 (10%)
Ollech et al. [24], APT, 2020	71 (only 48 patients [68%] received CyA, the rest received TC; 60 [85%] previously exposed to anti-TNF)	Continuous infusion 2–4 mg/kg/day (serum trough levels of 300–400 ng/mL). If response, switch to oral CyA (daily dose equivalent to twice the 24 h IV dose)	300 mg at weeks 0, 2, and 6, and then every 8 weeks. Could be prescribed every 4 weeks after induction (44% had dose escalation). Vedolizumab was started after a median of 29 days (IQR 16–44) from the initiation of the calcineurin inhibitors	30/71 (42%) – both considering CyA and TC induction	0/71 (0%)
Resál et al. [25], APT, 2020	13 (no description of the population)	4 mg/kg IV for 5 days followed by oral CyA until week 52 or until the occurrence of the first unbearable side effect	First infusion 2 weeks after CyA. No information about vedolizumab dose	0/13 (0%)	0/13 (0%)
Tarabar et al. [50], Inflamm Bowel Dis, 2022	17 (2 [12%] previously IFX-exposed)	2–4 mg/kg/day IV for 7 days (goal trough level of 300–400 ng/mL), followed by oral CyA (double the initial IV dose) over 8 weeks, then discontinued	300 mg at the start of oral CyA (week 0), then at weeks 2 and 6, followed by every 8 weeks	3/17 (18%)	0 (0%)

ASUC, acute severe ulcerative colitis; CyA, cyclosporin; IFX, infliximab; SAE, severe adverse event; TC, tacrolimus.

studies have a small follow-up of 2–4 weeks and there is scarce evidence on the long-term benefit of TC, particularly in reducing colectomy rates.

The Role of Janus Kinase Inhibitors in ASUC

Janus kinase (JAK) inhibitors like tofacitinib (OCTAVE), filgotinib (SELECTION), and upadacitinib (U-ACHIEVE and U-ACCOMPLISH) are rapidly acting oral small molecules that have been approved for use in UC. They have different selectivity, with tofacitinib acting mostly on JAK 1 and JAK 3 receptors and filgotinib and upadacitinib with a more selective inhibition of JAK 1 receptor [33–35]. Several characteristics make JAK inhibitors attractive drugs in this setting, namely, their rapid onset of action and rapid clinical response with significant reduction by day 3 of baseline stool frequency sub-score, total number of daily bowel movements, and rectal bleeding sub-score when compared with placebo [36]. Their short half-life also leads to a rapid clearance, which may be relevant in the case of foreseeing colectomy, with a theoretical benefit in reducing perioperative complications. Moreover, as small molecules, they are less susceptible to drug loss, due to hypoalbuminemia and colonic protein loss, when compared to biologics.

Table 2. CyA plus ustekinumab as a rescue therapy in steroid-refractory ASUC

Study	Sample size	CyA induction regimen	Ustekinumab maintenance regimen	Colectomy during follow-up	SAE
Ganzleben et al. [28], Ther Adv Gastroenterol, 2020	1 (previously exposed to adalimumab, IFX, vedolizumab, CyA combined with azathioprine and mercaptopurine, and tofacitinib)	2 mg/kg/day IV followed by oral CyA for a serum concentration of 250–300 ng/mL (discontinued at day 82)	Loading dose of 390 mg IV on day 6 of CyA; maintenance with subcutaneous ustekinumab (no reference to the interval of administration)	0/1 (0%)	0/1 (0%)
Shaffer et al. [29], ACG Case Reports J, 2021	2 (not previously exposed to IFX)	3 mg/kg/day IV (target level 300–400 ng/mL), followed by oral CyA discontinued at week 8	Loading dose of 390 mg IV on day 4 and loading dose of 260 mg IV on week 11 after CyA initiation (failure of bridging to vedolizumab); maintenance with subcutaneous ustekinumab (no reference to the interval of administration)	0/2 (0%)	0/2 (0%)
Veyrard et al. [27], Clin Gastroenterol Hepatol, 2022	10 (9 [90%] previously exposed to IFX)	2 mg/kg/day IV for 7 days (blood concentration between 150 and 250 ng/mL), followed by oral CyA withdrawn within the first 3 months after therapy initiation	Loading dose of 6 mg/kg, followed by 90 mg subcutaneously every 8 weeks	0/10 (0%)	0/10 (0%)

ASUC, acute severe ulcerative colitis; CyA, cyclosporin; SAE, severe adverse event.

Tofacitinib has been the JAK inhibitor most frequently described in case reports and in a few case series and retrospective case-control studies as a rescue therapy in ASUC (shown in Table 3). To date, the largest report on the use of tofacitinib in ASUC included 55 patients who received 10 mg of tofacitinib bid and demonstrated a colectomy-free survival of 78.9% (95% CI 68.5–90.9) and 73.6% (95% CI 61.9–87.3) at 3 and 6 months, respectively. Despite these promising results, this study has a major limitation of not having a comparison group [37].

A retrospective case-control study performed by Berinstein et al. [38] included 40 biologic-experienced patients admitted with ASUC treated with intravenous steroids and tofacitinib which were matched 1:3 to controls (n = 113) according to gender and date of admission. Using Cox regression analysis adjusted for disease severity, tofacitinib was protective against colectomy at 90 days (HR 0.28, 95% CI 0.10–0.81, p = 0.018), although this result was only significant for patients taking tofacitinib 10 mg tid (HR 0.11, 95% CI 0.02–0.56, p = 0.008) [38]. Despite being the larger study with a comparison group, it is important to highlight that this work only focused on the role of tofacitinib as an adjuvant of corticosteroid therapy in inducing remission in biologic-experienced patients hospitalized with ASUC and did not assess the role of tofacitinib as a rescue therapy for steroid non-responders. Namely, in the control group only 39.8% of the patients needed a rescue therapy, which means that most patients responded to steroids. Furthermore, some case reports and case series have also explored the possible role of tofacitinib as a second-line rescue therapy in patients who have failed IFX, with a colectomy-free survival at 6 months of 62.5% [39]. In a systematic review of 21 patients with ASUC, tofacitinib demonstrated an efficacy of 75% (3/4) as first-line therapy, 85.7% (12/14) as second-line therapy (steroid failure), and 66.6% (2/3) as third-line therapy [40]. In another more recent systematic review including 148 reported cases using tofacitinib as second-line treatment after steroid failure in previous IFX failures or third line

Table 3. Tofacitinib as a rescue therapy in steroid-refractory ASUC

Study	Sample size	Tofacitinib regimen	Colectomy during follow-up	SAE
Berinstein et al. [51], Clin Gastroenterol Hepatol, 2019	4 (2 [50%] prior IFX exposure)	10 mg tid for 9 doses at the same time of IV corticosteroid induction	1/4 (25%)	1/4 (25%)
Kotwani et al. [52], J Crohns Colitis, 2020	4 (100% prior IFX exposure)	10 mg bid (and 1 patient 15 mg bid after 10 days due to insufficient improvement) started 3–6 days after admission	0/4 (0%)	0/4 (0%)
Honap et al. [53], Inflamm Bowel Dis, 2020	7 (5 [71%] prior IFX exposure)	10 mg bid	4/7 (57%)	0/7 (0%)
Uzzan et al. [37], APT, 2021	55 (49% [89%] prior IFX failure)	10 mg bid started at a median of 3 days after admission (IQR 1–6)	15/55 (27%)	3/55 (5%)
Sedano et al. [54], Inflamm Bowel Dis, 2021	1 (100% prior IFX exposure)	10 mg bid	0/1 (0%)	0/1 (0%)
Berinstein et al. [38], Clin Gastroenterol Hepatol, 2021	40 (34 [85%] prior IFX failure)	10 mg bid or 10 mg tid for 9 doses followed by 10 mg twice daily. Only the dose 10 mg tid was protective for colectomy	6/40 (15%)	1/40 (2.5%)
Jena et al. [40], Inflamm Bowel Dis, 2021	4 (2 [50%] failed IFX as second-line rescue therapy in ASUC)	10 mg bid after CyA or IFX failure	1/4 (25%)	1/4 (25%)
Yang et al. [55], Inflamm Bowel Dis, 2021	1 (100% prior IFX exposure)	CyA 3 mg/kg/day IV + tofacitinib 10 mg bid, followed 1 month later by oral CyA 150 mg bid + tofacitinib 5 mg bid and after 6 months, CyA was discontinued	0/1 (0%)	0/1 (0%)
Gilmore et al. [43], J Crohns Colitis, 2023	5 (3 [60%] prior IFX exposure)	10 mg tid started at a median of 4 days after admission [IQR 3–8] and for a maximum of 14 days, following which the dose was reduced to 10 mg bid for a further 8 weeks. Then, 5 mg bid as maintenance therapy	1/5 (20%)	0/5 (0%)
Xiao et al. [39], Dig Dis Sci, 2022	8 (100% prior exposure to IFX, 3 [38%] during the same ASUC episode)	Five patients received 10 mg bid, while 3 patients received 10 mg tid for 3 days followed by 10 mg bid	2/8 (25%)	0/8 (0%)
Santos et al. [56], GE Port J Gastroenterol, 2021	2 (100% prior exposure to at least one anti-TNF)	10 mg bid	0/2 (0%)	0/2 (0%)

ASUC, acute severe ulcerative colitis; CyA, cyclosporin; IFX, infliximab; SAE, severe adverse event.

after sequential steroid and IFX or CyA failure, the 30-, 90-, and 180-day colectomy-free survival was 85%, 86%, and 69%, respectively [41]. Also, the ORCHID trial has recently demonstrated no differences on the efficacy and safety of tofacitinib for induction of remission in moderately active UC when compared with oral prednisolone [42]. Although there do not seem to exist significant safety issues with the use of tofacitinib in ASUC, some authors highlight possible concerns regarding a heightened risk of thrombosis in this setting and infectious risk, particularly herpes zoster infection [40, 41].

There was a significant heterogeneity across published studies in the dose of tofacitinib used to induce remission ranging from 20 to 30 mg/day in two to three divided daily doses. The study by Berinstein et al. [38] raised the possibility that only higher daily doses of 10 mg tid were effective in reducing the risk of colectomy [38]. Larger, prospective, RCTs are needed to clarify the safest, optimal dose, frequency, and duration of JAK inhibitor therapy in ASUC. In the meantime, it should be well noted that the use of JAK inhibitors in patients with ASUC that have previously

failed IFX and are not responding to intravenous steroids is off-label, and probably should be reserved for referral centres with expertise in managing these patients.

One study (TRIUMPH trial, NCT04925973) is currently ongoing and is recruiting patients with primary non-response or secondary loss of response to immunomodulators, anti-TNFα, anti-integrin, or anti-interleukin therapies or non-response after 3–7 days of intravenous steroids, which will be assigned to receive 10 mg bid of tofacitinib (single group assignment), and the primary outcome will be clinical response at day 7. One further trial (TOCASU trial, NCT05112263) expected to start recruiting soon intends to compare CyA with oral tofacitinib (10 mg tid for 3 days, followed by 10 mg bid for 8 weeks and then 5 mg bid until week 14) as first-line rescue therapies in steroid-refractory ASUC. These studies will hopefully provide further evidence on the role of tofacitinib as primary or sequential salvage therapy in ASUC.

Although with only very few case reports published, upadacitinib has also been suggested as a salvage therapy in patients with steroid-refractory ASUC, with the first study demonstrating that in 6 patients with prior loss of response to IFX, it allowed a colectomy-free rate of 83% after a follow-up of 16 weeks [43]. In a second report including 4 patients, only 1 patient needed colectomy, while half of them achieved steroid-free clinical and endoscopic remission after 3 months [44]. Similar to CyA, the efficacy of JAK inhibitors in IFX-experienced patients can only be extrapolated from case reports and more robust evidence is needed in the setting.

Treatment in IFX-Experienced ASUC Patients: Which Way to Go?

The widespread use of IFX as a primary salvage therapy in ASUC, and for those with moderate-to-severe UC unresponsive to standard treatment, has increased the number of individuals previously exposed to anti-TNF drugs. This growing cohort, along with patients exposed to other biologics and small molecules, poses a challenge in managing medical therapy for ASUC admissions. Previous studies have shown that patients with prior anti-TNF or thiopurine treatment, Clostridioides difficile infection, and high C-reactive protein or low albumin levels face a high risk of colectomy within a year [45]. Despite these complexities, avoiding emergent colectomy remains a goal due to its association with significantly higher mortality and morbidity [46]. However, the risks of emergent surgery need careful consideration in comparison with the risks associated with various immunosuppressive therapies and possible surgery delay due to prolonged medical therapy, contributing to increased surgical complications [47, 48]. A multidisciplinary approach, including timely assessment, surgeon consultation, and early nutritional evaluation, is recommended. Enteral nutrition should be the preferred choice, and recent studies propose a role for exclusive enteral nutrition as an adjuvant to corticosteroid therapy [49].

Based on the current revised data, two medical approaches may be considered for steroid-refractory ASUC patients who have lost response to IFX in the past. The first is to induce remission with CyA and then transition to another biologic such as vedolizumab or ustekinumab, and the second is to use tofacitinib for both induction and maintenance. Due to the lack of evidence, the choice between these strategies should rely on local policies, physician's experience, and patient's medical history. For example, in patients who have been exposed to ustekinumab and/or vedolizumab in addition to IFX, using a CyA-based strategy may be limited due to the lack of an adequate maintenance therapy, making tofacitinib a potential option. A proposed algorithm of approach is shown in Figure 1. However, since evidence is limited, caution should be exercised when making this choice. The TOCASU trial's results, comparing the efficacy of CyA and tofacitinib for steroid-refractory ASUC patients, will be relevant in this context. Other relevant unanswered questions include determining the preferred dose regimen for each drug, such as finding the most effective serum concentration for CyA and identifying the best dosing regimen for tofacitinib. Additionally, it is unclear whether tofacitinib should be initiated alongside steroids as a pre-emptive measure in patients who have failed IFX and it would be intriguing for future research to explore the prospect of initiating salvage therapy directly with this rapidly acting small molecules, circumventing the use of high-dose steroids, which can be associated with additional surgical risks. Furthermore, there is uncertainty about when it is appropriate to transition from intravenous CyA to oral CyA and when to begin maintenance therapy with CyA.

It is never enough to emphasize that ASUC is a serious condition with the potential for serious complications and that surgeons should be involved from the first day in complex decisions such as rescue therapy. Hence, it is imperative to handle IFX-experienced, steroid-refractory ASUC patients in specialized centres, under the guidance

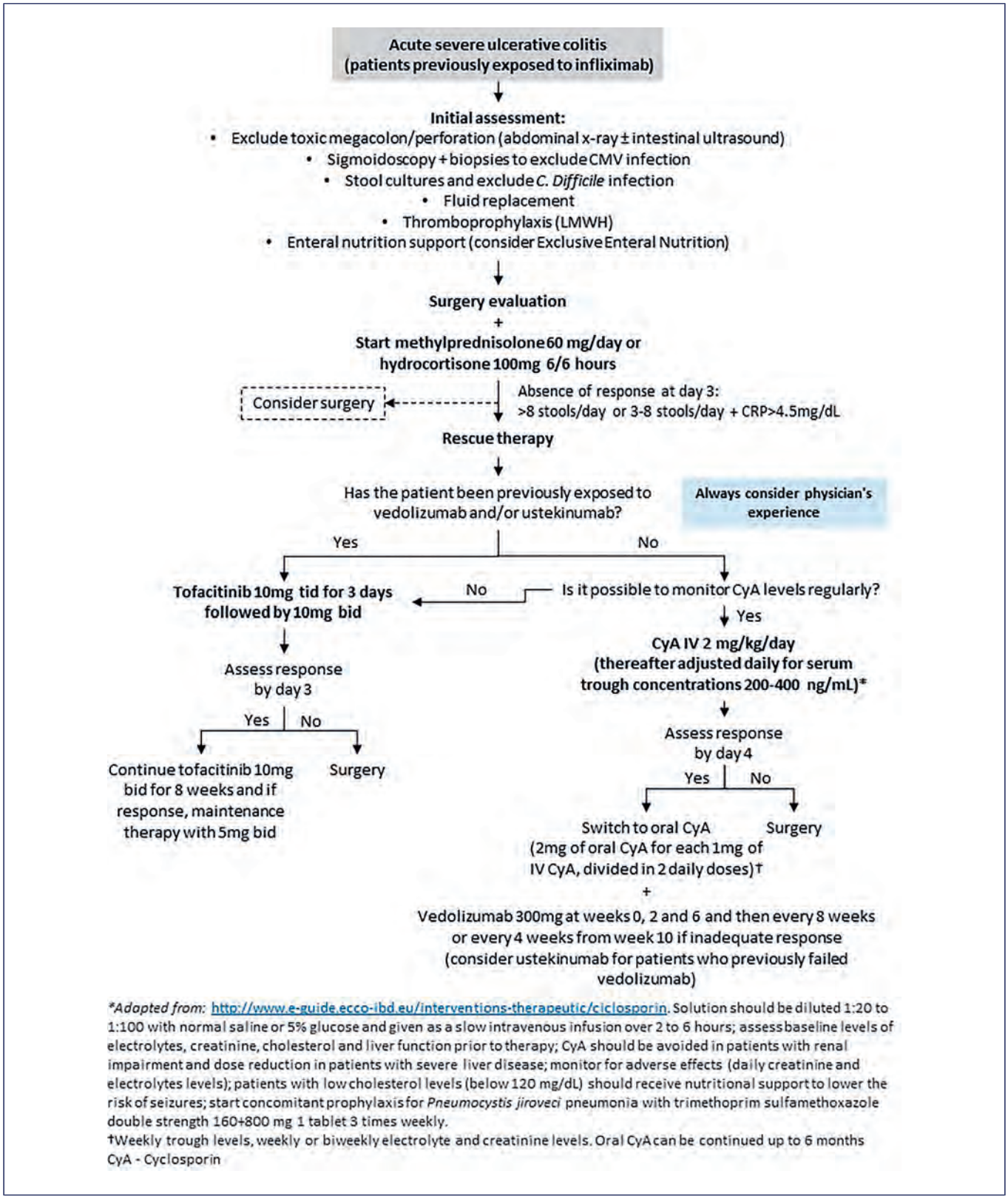


Fig. 1. Proposed algorithm for approaching steroid-refractory ASUC in patients previously exposed to IFX.

of a multidisciplinary medical-surgical team. Decisions should be collaboratively made with the patient, tailoring the approach to define the optimal treatment strategy for each unique case. The overarching objective is not merely to preserve the patient's colon but, more significantly, to safeguard and enhance the patient's overall well-being and life.

Conclusion

ASUC patients who are refractory to intravenous steroids and who have previously been exposed to IFX are a difficult-to-treat group of patients whose incidence has been increasing. Although the emergence of new biological and small molecule therapies is promising and has allowed the development of different salvage therapy algorithms, evidence is still scarce. Further RCTs are needed to define the best treatment for this group of patients, and surgery should always be considered.

Conflict of Interest Statement

Carolina Palmela has received honoraria fees from Janssen, Laboratórios Vitória, and Sandoz. Joana Torres has received advisory board/speaker fees from AbbVie, Janssen, Pfizer, and Sandoz, and grant support from AbbVie and Janssen.

Funding Sources

No funding was provided.

Author Contributions

Joana Revés and Carolina Palmela contributed to the manuscript design. Joana Revés, Catarina Frias-Gomes, Lídia Roque Ramos, Joana Torres, and Carolina Palmela wrote the manuscript. Ana Catarina Bravo, Catarina Neto Nascimento, Bárbara Morão, and Luísa Glória provided significant revisions to the manuscript. All authors critically revised the manuscript. All authors have approved the final version of the manuscript.

References

1 Dinesen LC, Walsh AJ, Protic MN, Heap G, Cummings F, Warren BF, et al. The pattern and outcome of acute severe colitis. J Crohns Colitis. 2010;4(4):431–7.
2 Dong C, Metzger M, Holsbø E, Perduca V, Carbonnel F. Systematic review with meta-analysis: mortality in acute severe ulcerative colitis. Aliment Pharmacol Ther. 2020;51(1):8–33.
3 Turner D, Walsh CM, Steinhart AH, Griffiths AM. Response to corticosteroids in severe ulcerative colitis: a systematic review of the literature and a meta-regression. Clin Gastroenterol Hepatol. 2007;5(1):103–10.
4 Travis S, Satsangi J, Lémann M. Predicting the need for colectomy in severe ulcerative colitis: a critical appraisal of clinical parameters and currently available biomarkers. Gut. 2011;60(1):3–9.
5 Worley G, Almoudaris A, Bassett P, Segal J, Akbar A, Ghosh S, et al. Colectomy rates for ulcerative colitis in England 2003-2016. Aliment Pharmacol Ther. 2021;53(4):484–98.
6 van der Valk ME, Mangen MJJ, Leenders M, Dijkstra G, van Bodegraven AA, Fidder HH, et al. Healthcare costs of inflammatory bowel disease have shifted from hospitalisation and surgery towards anti-TNFα therapy: results from the COIN study. Gut. 2014;63(1):72–9.
7 Truelove SC, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. Br Med J. 1955;2(4947):1041–8.
8 Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, et al. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N Engl J Med. 1994;330(26):1841–5.
9 Van Assche G, D'Haens G, Noman M, Vermeire S, Hiele M, Asnong K, et al. Randomized, double-blind comparison of 4 mg/kg versus 2 mg/kg intravenous cyclosporine in severe ulcerative colitis. Gastroenterology. 2003;125(4):1025–31.
10 Järnerot G, Hertervig E, Friis-Liby I, Blomquist L, Karlén P, Grännö C, et al. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. Gastroenterology. 2005;128(7):1805–11.
11 Laharie D, Bourreille A, Branche J, Allez M, Bouhnik Y, Filippi J, et al. Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial. Lancet. 2012;380(9857):1909–15.
12 Williams JG, Alam MF, Alrubaiy L, Arnott I, Clement C, Cohen D, et al. Infliximab versus ciclosporin for steroid-resistant acute severe ulcerative colitis (CONSTRUCT): a mixed methods, open-label, pragmatic randomised trial. Lancet Gastroenterol Hepatol. 2016;1(1):15–24.
13 Brandse JF, van den Brink GR, Wildenberg ME, van der Kleij D, Rispens T, Jansen JM, et al. Loss of infliximab into feces is associated with lack of response to therapy in patients with severe ulcerative colitis. Gastroenterology. 2015;149(2):350–5.e2.
14 Brandse JF, Mathôt RA, van der Kleij D, Rispens T, Ashruf Y, Jansen JM, et al. Pharmacokinetic features and presence of antidrug antibodies associate with response to infliximab induction therapy in patients with moderate to severe ulcerative colitis. Clin Gastroenterol Hepatol. 2016;14(2):251–8.e1–2.
15 Nalagatla N, Falloon K, Tran G, Borren NZ, Avalos D, Luther J, et al. Effect of accelerated infliximab induction on short- and long-term outcomes of acute severe ulcerative colitis: a retrospective multicenter study and meta-analysis. Clin Gastroenterol Hepatol. 2019;17(3):502–9.e1.
16 Sebastian S, Myers S, Argyriou K, Martin G, Los L, Fiske J, et al. Infliximab induction regimens in steroid-refractory acute severe colitis: a multicentre retrospective cohort study with propensity score analysis. Aliment Pharmacol Ther. 2019;50(6):675–83.
17 Shah SC, Naymagon S, Cohen BL, Sands BE, Dubinsky MC. There is significant practice pattern variability in the management of the hospitalized ulcerative colitis patient at a tertiary care and IBD referral center. J Clin Gastroenterol. 2018;52(4):333–8.
18 Ordás I, Domènech E, Mañosa M, García-Sánchez V, Iglesias-Flores E, Peñalva M, et al. Long-term efficacy and safety of cyclosporine in a cohort of steroid-refractory acute severe ulcerative colitis patients from the ENEIDA registry (1989-2013): a Nationwide Multicenter Study. Am J Gastroenterol. 2017;112(11):1709–18.
19 Laharie D, Bourreille A, Branche J, Allez M, Bouhnik Y, Filippi J, et al. Long-term outcome of patients with steroid-refractory acute severe UC treated with ciclosporin or infliximab. Gut. 2018;67(2):237–43.

20 Narula N, Marshall JK, Colombel JF, Leontiadis GI, Williams JG, Muqtadir Z, et al. Systematic review and meta-analysis: infliximab or cyclosporine as rescue therapy in patients with severe ulcerative colitis refractory to steroids. Am J Gastroenterol. 2016;111(4):477–91.
21 García-López S, Gomollón-García F, Pérez-Gisbert J. Cyclosporine in the treatment of severe attack of ulcerative colitis: a systematic review. Gastroenterol Hepatol. 2005;28(10):607–14.
22 Weisshof R, Ollech JE, El Jurdi K, Yvellez OV, Cohen RD, Sakuraba A, et al. Ciclosporin therapy after infliximab failure in hospitalized patients with acute severe colitis is effective and safe. J Crohns Colitis. 2019;13(9):1105–10.
23 Gisbert JP, García MJ, Chaparro M. Rescue therapies for steroid-refractory acute severe ulcerative colitis: a review. J Crohns Colitis. 2023;17(6):972–99.
24 Ollech JE, Dwadasi S, Rai V, Peleg N, Normatov I, Israel A, et al. Efficacy and safety of induction therapy with calcineurin inhibitors followed by vedolizumab maintenance in 71 patients with severe steroid-refractory ulcerative colitis. Aliment Pharmacol Ther. 2020;51(6):637–43.
25 Resál T, Pigniczki D, Szántó K, Rutka M, Farkas K, Molnar T. Letter: ciclosporin and vedolizumab for steroid-refractory ulcerative colitis. Aliment Pharmacol Ther. 2020;52(3):578–9.
26 Pellet G, Stefanescu C, Carbonnel F, Peyrin-Biroulet L, Roblin X, Allimant C, et al. Efficacy and safety of induction therapy with calcineurin inhibitors in combination with vedolizumab in patients with refractory ulcerative colitis. Clin Gastroenterol Hepatol. 2019;17(3):494–501.
27 Veyrard P, Pellet G, Laharie D, Nachury M, Juillerat P, Roblin X. Efficacy of induction therapy with calcineurin inhibitors in combination with ustekinumab for acute severe ulcerative colitis. Clin Gastroenterol Hepatol. 2023;21(5):1354–5.e2.
28 Ganzleben I, Geppert C, Osaba L, Hirschmann S, Nägel A, Glück C, et al. Successful cyclosporin and ustekinumab combination therapy in a patient with severe steroid-refractory ulcerative colitis. Therap Adv Gastroenterol. 2020;13:1756284820954112.
29 Shaffer SR, Traboulsi C, Krugliak Cleveland N, Rubin DT. Combining cyclosporine with ustekinumab in acute severe ulcerative colitis. ACG Case Rep J. 2021;8(5):e00604.
30 Ogata H, Matsui T, Nakamura M, Iida M, Takazoe M, Suzuki Y, et al. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. Gut. 2006;55(9):1255–62.
31 Komaki Y, Komaki F, Ido A, Sakuraba A. Efficacy and safety of tacrolimus therapy for active ulcerative colitis: a systematic review and meta-analysis. J Crohns Colitis. 2016;10(4):484–94.
32 Komaki Y, Komaki F, Micic D, Yamada A, Suzuki Y, Sakuraba A. Pharmacologic therapies for severe steroid refractory hospitalized ulcerative colitis: a network meta-analysis. J Gastroenterol Hepatol. 2017;32(6):1143–51.
33 Sandborn WJ, Su C, Sands BE, D'Haens GR, Vermeire S, Schreiber S, et al. Tofacitinib as induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2017;376(18):1723–36.
34 Feagan BG, Danese S, Loftus EV Jr, Vermeire S, Schreiber S, Ritter T, et al. Filgotinib as induction and maintenance therapy for ulcerative colitis (SELECTION): a phase 2b/3 double-blind, randomised, placebo-controlled trial. Lancet. 2021;397(10292):2372–84.
35 Danese S, Vermeire S, Zhou W, Pangan AL, Siffledeen J, Greenbloom S, et al. Upadacitinib as induction and maintenance therapy for moderately to severely active ulcerative colitis: results from three phase 3, multicentre, double-blind, randomised trials. Lancet. 2022;399(10341):2113–28.
36 Hanauer S, Panaccione R, Danese S, Cheifetz A, Reinisch W, Higgins PDR, et al. Tofacitinib induction therapy reduces symptoms within 3 Days for patients with ulcerative colitis. Clin Gastroenterol Hepatol. 2019;17(1):139–47.
37 Uzzan M, Bresteau C, Laharie D, Stefanescu C, Bellanger C, Carbonnel F, et al. Tofacitinib as salvage therapy for 55 patients hospitalised with refractory severe ulcerative colitis: a GETAID cohort. Aliment Pharmacol Ther. 2021;54(3):312–9.
38 Berinstein JA, Sheehan JL, Dias M, Berinstein EM, Steiner CA, Johnson LA, et al. Tofacitinib for biologic-experienced hospitalized patients with acute severe ulcerative colitis: a retrospective case-control study. Clin Gastroenterol Hepatol. 2021;19(10):2112–20.e1.
39 Xiao Y, Benoit N, Sedano R, Jairath V, Narula N, McCurdy JD, et al. Effectiveness of tofacitinib for hospitalized patients with acute severe ulcerative colitis: case series. Dig Dis Sci. 2022;67(11):5213–9.
40 Jena A, Mishra S, Sachan A, Singh H, Singh AK, Sharma V. Tofacitinib in acute severe ulcerative colitis: case series and a systematic review. Inflamm Bowel Dis. 2021;27(9):e101–3.
41 Steenholdt C, Ovesen PD, Brynskov J, Seidelin JB. Tofacitinib for acute severe ulcerative colitis: a systematic review. J Crohns Colitis. 2023;17(8):1354–63.
42 Singh A, Midha V, Kaur K, Mahajan R, Singh D, Kaur R, et al. Tofacitinib versus oral prednisolone for induction of remission in moderately active ulcerative colitis (ORCHID): a prospective, open-label, randomized, pilot study. J Crohns Colitis. 2023:jjad153.
43 Gilmore R, Tan L, Fernandes R, An YK, Begun J. Upadacitinib salvage therapy for infliximab experienced patients with acute severe ulcerative colitis. J Crohns Colitis. 2023;17(12):2033–6.
44 Zinger CH, Ringel Y, Eitan M, Openhaim M, Kayless H, Stein A, et al. Upadacitinib for acute severe ulcerative colitis. Inflamm Bowel Dis. 2023;29(10):1667–9.
45 Le Baut G, Kirchgesner J, Amiot A, Lefevre JH, Chafai N, Landman C, et al. A scoring system to determine patients' risk of colectomy within 1 Year after hospital admission for acute severe ulcerative colitis. Clin Gastroenterol Hepatol. 2021;19(8):1602–10.e1.
46 Roberts SE, Williams JG, Yeates D, Goldacre MJ. Mortality in patients with and without colectomy admitted to hospital for ulcerative colitis and Crohn's disease: record linkage studies. BMJ. 2007;335(7628):1033.
47 Teeuwen PH, Stommel MW, Bremers AJ, van der Wilt GJ, de Jong DJ, Bleichrodt RP. Colectomy in patients with acute colitis: a systematic review. J Gastrointest Surg. 2009;13(4):676–86.
48 Randall J, Singh B, Warren BF, Travis SP, Mortensen NJ, George BD. Delayed surgery for acute severe colitis is associated with increased risk of postoperative complications. Br J Surg. 2010;97(3):404–9.
49 Sahu P, Kedia S, Vuyyuru SK, Bajaj A, Markandey M, Singh N, et al. Randomised clinical trial: exclusive enteral nutrition versus standard of care for acute severe ulcerative colitis. Aliment Pharmacol Ther. 2021;53(5):568–76.
50 Tarabar D, El Jurdi K, Traboulsi C, Yvellez O, Milenkovic Z, Petrovic S, et al. A prospective trial with long term follow-up of patients with severe, steroid-resistant ulcerative colitis who received induction therapy with cyclosporine and were maintained with vedolizumab. Inflamm Bowel Dis. 2022;28(10):1549–54.
51 Berinstein JA, Steiner CA, Regal RE, Allen JI, Kinnucan JAR, Stidham RW, et al. Efficacy of induction therapy with high-intensity tofacitinib in 4 patients with acute severe ulcerative colitis. Clin Gastroenterol Hepatol. 2019;17(5):988–90.e1.
52 Kotwani P, Terdiman J, Lewin S. Tofacitinib for rescue therapy in acute severe ulcerative colitis: a real-world experience. J Crohns Colitis. 2020;14(7):1026–8.
53 Honap S, Pavlidis P, Ray S, Sharma E, Anderson S, Sanderson JD, et al. Tofacitinib in acute severe ulcerative colitis-a real-world tertiary center experience. Inflamm Bowel Dis. 2020;26(11):e147–9.
54 Sedano R, Jairath V. Tofacitinib induced long-term clinical, endoscopic, and histological remission as a 6th line agent in medically refractory ulcerative colitis. Inflamm Bowel Dis. 2021;27(3):e35–6.
55 Yang Q, Chen L, Feng L, Liu C, Fang L, Liu Z, et al. Success of cyclosporin and tofacitinib combination therapy in a patient with severe steroid-refractory ulcerative colitis. Inflamm Bowel Dis. 2021;27(12):e157–8.
56 Santos S, Gamelas V, Saraiva R, Simões G, Saiote J, Ramos J. Tofacitinib: an option for acute severe ulcerative colitis? GE Port J Gastroenterol. 2021;29(2):132–4.

GE – Portuguese Journal of Gastroenterology

Research Article

GE Port J Gastroenterol 2024;31:325–337
DOI: 10.1159/000534740

Received: June 13, 2023
Accepted: October 12, 2023
Published online: November 15, 2023

COVID-19 Vaccination in Liver Cirrhosis: Safety and Immune and Clinical Responses

Inês Canha[a, b] Mário Jorge Silva[a, b] Maria Azevedo Silva[c] Mara Sarmento Costa[d] Rita Ornelas Saraiva[a] André Ruge[c] Mariana Verdelho Machado[e, f] Catarina Sousa Félix[g] Bárbara Morão[h] Pedro Narra Figueiredo[d, i] Milena Mendes[a] Carina Leal[c] Filipe Calinas[a]

[a]Gastroenterology Department, Centro Hospitalar Universitário de Lisboa Central, Lisbon, Portugal; [b]NOVA Medical School, Universidade NOVA de Lisboa, Lisbon, Portugal; [c]Gastroenterology Department, Centro Hospitalar de Leiria, Leiria, Portugal; [d]Gastroenterology Department, Centro Hospitalar Universitário de Coimbra, Coimbra, Portugal; [e]Gastroenterology Department, Hospital de Vila Franca de Xira, Vila Franca de Xira, Portugal; [f]Faculty of Medicine, Universidade de Lisboa, Lisbon, Portugal; [g]Gastroenterology Department, Centro Hospitalar Lisboa Ocidental, Lisbon, Portugal; [h]Gastroenterology Department, Hospital Beatriz Ângelo, Lisbon, Portugal; [i]Faculty of Medicine, Universidade de Coimbra, Coimbra, Portugal

Keywords
COVID-19 · Liver cirrhosis · Vaccine · Infection · Antibodies

Abstract

Introduction: Three years after the beginning of the SARS-CoV-2 pandemic, the safety and efficacy of COVID-19 vaccination in liver cirrhosis (LC) patients remain controversial. We aimed to study the safety, immunological, and clinical responses of LC patients to COVID-19 vaccination. ***Methods:*** Prospective multicentric study in adults with LC eligible for COVID-19 vaccination, without prior known infection. Patients were followed up until the timing of a booster dose, SARS-CoV-2 infection, or death. Spike-protein immunoglobulin G antibody titers for SARS-CoV-2 at 2 weeks, 3 months, and 6 months postvaccination were assessed. Antibody titers <33.8 binding antibody units (BAU)/mL were considered seronegative and <200 BAU/mL suboptimal. Postvaccination infection and its severity were registered. ***Results:*** We included 124 LC patients, 81% males, mean aged 61 ± 10 years, with a mean follow-up of 221 ± 26 days. Alcohol was the most common (61%) cause of cirrhosis, and 7% were under immunosuppressants for autoimmune hepatitis; 69% had portal hypertension, 42% had a previous decompensation, and 21% had a Child-Pugh-Turcotte score of B/C. The type of vaccine administrated was BNT162b2 (*n* = 59, 48%), ChAdOx1nCoV-19 (*n* = 45, 36%), mRNA-1273 (*n* = 14, 11%), and Ad26.COV2.S (*n* = 6, 5%). Eighteen percent of the patients reported adverse events after vaccination, none serious. Median [Q1; Q3] antibody titers were 1,185 [280; 2,080] BAU/mL at 2 weeks, 301 [72; 1,175] BAU/mL at 3 months, and 192 [49; 656] BAU/mL at 6 months. There were seronegative and suboptimal antibody responses in 8% and 23% of the patients at 2 weeks, 16% and 38% at 3 months, and 22% and 48% at 6 months. Older age and adenovirus vector vaccines were the only factors associated with

karger@karger.com
www.karger.com/pjg

Correspondence to:
Inês Canha, m.inescanha@gmail.com

seronegative and suboptimal responses at 2 weeks and 3 months ($p < 0.05$) in a multivariable logistic regression analysis. Eleven patients (9%) were infected with SARS-CoV-2 during follow-up (3.8–6.6 months postvaccination), all with mild disease. There were no differences regarding the type of vaccine, and 73% had antibody titers >200 BAU/mL at 3 months. ***Conclusion:*** COVID-19 vaccines in patients with LC were safe, without serious adverse events. The humoral and clinical responses were similar to the reported for the general population. Humoral response was adversely impacted by older age and adenovirus vector vaccines and unrelated to the liver disease severity.

Vacinação COVID-19 na cirrose hepática: segurança e respostas imunológica e clínica

Palavras Chave
COVID-19 · Cirrose hepática · Vacina · Infeção · Anticorpos

Resumo
Introdução: Três anos após o início da pandemia SARS-CoV-2, a segurança e eficácia da vacinação COVID-19 em doentes com cirrose hepática (CH) permanecem controversas. Pretendemos avaliar a segurança, respostas imunológica e clínica de doentes com CH às vacinas contra a COVID-19. ***Métodos:*** Estudo prospetivo multicêntrico em adultos com CH elegíveis para vacinação contra a COVID-19, sem infeção prévia conhecida. Os doentes foram acompanhados até ao momento da dose de reforço, infeção SARS-CoV-2 ou falecimento. Avaliámos os títulos de anticorpos IgG da proteína-*Spike* SARS-CoV-2 às 2 semanas, 3 meses e 6 meses. Títulos de anticorpos <33.8 BAU/mL foram considerados seronegativos e <200 BAU/mL subótimos. A ocorrência de infeção pós-vacinação e respetiva gravidade foram registadas. ***Resultados:*** Incluímos 124 doentes com CH, 81% homens, com idade média de 61 ± 10 anos e um seguimento médio de 221 ± 26 dias. A causa mais prevalente de cirrose foi o álcool (61%) e 7% dos doentes faziam terapêutica imunossupressora por hepatite autoimune. Existiam sinais de hipertensão portal em 69%, descompensação prévia em 42% e classificação de *Child-Pugh-Turcotte* B/C em 21%. O tipo de vacina administrada foi: BNT162b2 ($n = 59$, 48%), ChAdOx1nCoV-19 ($n = 45$, 36%), mRNA-1273 ($n = 14$, 11%) e Ad26.COV2.S ($n = 6$, 5%). Foram reportados efeitos adversos pós-vacinação em 18% dos participantes, nenhum deles grave. Os títulos medianos [Q1; Q3] de anticorpos foram 1.185 [280; 2.080] BAU/mL às 2 semanas, 301 [72; 1.175] BAU/mL aos 3 meses e 192 [49; 656] BAU/mL aos 6 meses. Observámos respostas humorais seronegativas e subótimas em 8% e 23% dos doentes às 2 semanas, 16% e 38% aos 3 meses e 22% e 48% aos 6 meses. A idade avançada e vacinas de vetor de adenovírus foram os únicos fatores associados a respostas seronegativas e subótimas às 2 semanas e 3 meses ($p < 0.05$) em análise de regressão logística multivariada. Onze doentes (9%) desenvolveram infeção SARS-CoV-2 durante o seguimento (3.8–6.6 meses pós vacinação), todos com doença ligeira. Não observámos diferenças relativamente ao tipo de vacina, apresentando 73% deles títulos de anticorpos >200 BAU/mL aos 3 meses. ***Conclusões:*** A vacinação contra a COVID-19 em doentes com CH foi segura, sem efeitos adversos graves. As respostas humoral e clínica foram semelhantes às reportadas na população geral. A resposta humoral foi afetada negativamente pela idade avançada e vacinas de vetor de adenovírus e não apresentou relação com a gravidade da doença hepática.

Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for the novel coronavirus disease 2019 (COVID-19), first reported in December 2019 in Wuhan, China, and later becoming a global pandemic [1]. Since the onset of the COVID-19 pandemic, major efforts were made in the development of a SARS-CoV-2 vaccine, leading to the fast development of safe and highly effective vaccines.

The immunological response to vaccines leads to the release of inflammatory cytokines, antibodies targeted to the virus' spike protein, blocking its entrance in host cells, and the activation of Th1 lymphocytes, leading to the expansion of CD4+ and CD8+ T cells and the development of memory T cells [2–4]. The primary immunological endpoint of COVID-19 vaccines was the induction of neutralizing antibodies against the SARS-CoV-2 spike protein [4], which correlate with binding anti-Spike antibody titers and are detected in 90% of the seroconverters [5]. Clinical endpoints of vaccine efficacy trials were assessed through virologically confirmed symptomatic

SARS-CoV-2 infection and virologically confirmed SARS-CoV-2 infection with symptoms classified as severe [6].

The Pfizer/Comirnaty® [BNT162b2 mRNA], Moderna/Spikevax® [mRNA-1273], and AstraZeneca/Vaxzevria® [ChAdOx1-nCoV-19] vaccines showed excellent safety profiles and good efficacy in preventing symptomatic COVID-19 (62–95%) in the general population, but data were lacking regarding their effect in certain types of patients in clinical trials, such as those with chronic liver disease (CLD) [7]. Patients with CLD were excluded from the ChAdOx1-nCoV-19 trials and were not specifically identified in trials from the other two mRNA vaccines. Also, individuals under chronic immunosuppressive treatment such as autoimmune hepatitis (AIH) patients or liver transplant recipients were excluded from all the trials [7]. Similarly, in Janssen/Jcovden® [Ad26.COV2.S] trials, only healthy individuals or a very small proportion of patients with CLD were included [8].

The innate and adaptive immune system responses are dysregulated in patients with CLD and may be further worsened by the use of immunosuppressant drugs in AIH [9, 10]. Due to the greater incidence and severity of infections in patients with liver cirrhosis (LC), vaccination against influenza, *Streptococcus pneumoniae*, hepatitis A virus, and hepatitis B virus is recommended. However, the durability of humoral immunity after influenza and pneumococcal vaccination is reduced [7], and these patients have lower rates of seroconversion in hepatitis A virus and hepatitis B virus vaccination [9]. Similarly, in the case of SARS-CoV-2 infection, patients with liver disease were expected to have an attenuated response to vaccination against COVID-19 [9, 10]. This response could be further affected by the cause and staging of cirrhosis, the use of certain medications such as immunosuppressants and patients' comorbidities. In addition, concerns regarding adverse reactions to COVID-19 vaccination in this vulnerable population, such as the risk of vaccine-triggered immune-mediated hepatitis and vaccine-induced thrombotic thrombocytopenia, were raised [11].

Furthermore, patients with LC who develop SARS-CoV-2 infection are associated with a more severe course of disease with worse clinical outcomes, when compared to non-cirrhotic patients [12, 13], making this susceptible population a priority for immunization and raising concerns regarding these patients' adequate protection. SARS-CoV-2 infection was associated with cirrhosis decompensation in 46% of the cases, with greater rates of decompensation, hospitalization, and mortality in patients with more advanced liver disease [12].

To conclude, previously available trials and real-life data regarding immunological and clinical efficacy of COVID-19 vaccination in patients with LC were scarce, raising concerns regarding their possibly lower immunological response to vaccines and worse clinical outcomes. Previously published data on these topics have shown conflicting results. While similar seroconversion rates have been described [14], other studies reported lower antibody titers in LC patients [15, 16]. Also, some authors have found similar infection rates after vaccination [17], while others saw a delayed reduction on the incidence of SARS-CoV-2 infection after vaccination between patients with and without LC [18]. Although most of the general population is now immunized either through vaccination or infection, the risk of spreading of new and more pathogenic variants can become a significant burden to healthcare systems and have particularly harmful consequences in vulnerable populations like LC patients. Our goals were to evaluate the safety, immunological, and clinical responses of patients with LC to COVID-19 vaccination and assess group differences regarding demographic, clinical, and vaccine-related factors.

Methods

Study Design and Patient Selection

We conducted a multicentric observational prospective study in adult patients with LC regularly followed in one of six hospitals in Portugal, eligible for vaccination against COVID-19 at the time of enrollment, with a 12-month follow-up after vaccination. Follow-up would be interrupted earlier in case of an additional booster dose of the vaccine, SARS-CoV-2 infection, or death. The introduction of a universal booster dose in our country during the study period led to follow-up termination before the 12-month timepoint in all patients.

Patients eligible for the study were adult subjects with a diagnosis of LC either confirmed by liver biopsy or through unequivocal clinical, biochemical, radiological, transient elastography, and/or endoscopic features of cirrhosis. Exclusion criteria were (1) contraindications for the COVID-19 vaccination program, (2) full vaccination before recruitment, (3) previously documented COVID-19 infection (either through a positive SARS-CoV-2 nucleic acid amplification test, rapid antigen test, or antibody measurement), (4) human immunodeficiency virus infection, and (5) treatment with immunosuppressant drugs for conditions other than AIH, to minimize confounding related to immunosuppression and lower vaccine responses [19].

All the patients enrolled in the study provided informed consent, in accordance with the tenets of the Declaration of Helsinki. The study was approved by the Ethics Committees of all the hospitals involved.

Patient Assessment and Follow-Up

Patients were assessed for eligibility on a regular hospital observation in six different hospitals in Portugal between May and August 2021. If they agreed to participate, the physician filled in a questionnaire with their baseline characteristics at the time of recruitment, including demographic, clinical, and complementary diagnostic test information. Patients were described regarding their age, gender, cause of LC, previous history of cirrhosis decompensation, features of portal hypertension, Child-Pugh-Turcotte (CPT) and Model for End-stage Liver Disease (MELD-Na) scores, important comorbidities, and current medication.

Patients were followed until completing COVID-19 vaccination (defined by the administration of 2 doses of the Pfizer/Comirnaty® [BNT162b2], AstraZeneca/Vaxzevria® [ChAdOx1nCoV-19], or Moderna/Spikevax® [mRNA-1273] vaccines or one dose of the Janssen/Jcovden® [Ad26.COV2.S] vaccine) through scheduled appointments, calls, or by regularly consulting the national healthcare data platform, where information regarding the timing and type of vaccine administered was provided. By this time, the vaccination date and type were recorded, and disease staging was reassessed and updated. Afterward, either by scheduled appointments or phone calls, patients were assessed at different timepoints: 2 weeks, 3 months, 6 months, and 12 months after completing vaccination, and/or until being given a booster dose of the vaccine, developing SARS-CoV-2 infection, or death.

Our main goals were to assess the safety and immunological and clinical efficacy of COVID-19 vaccination in patients with LC. Safety was ascertained through patients' self-reported data on adverse events following vaccination. Efficacy was measured both through humoral response (induction of immunoglobulin G [IgG]-binding antibodies against the SARS-CoV-2 spike protein) and clinical response (virologically confirmed SARS-CoV-2 infection and its severity). The severity of SARS-CoV-2 infection was defined according to the World Health Organization (WHO) Living Guidance [20] into mild (symptomatic disease without evidence of viral pneumonia or hypoxia), moderate (clinical signs of pneumonia but oxygen saturation [SpO2] ≥90% on room air), severe (clinical signs of pneumonia plus one of the following: respiratory rate >30 breaths/min, severe respiratory distress, or SpO2 <90% on room air), and critical (acute respiratory distress syndrome).

Patients were given a pseudoanonymized code and instructed to go to a partner laboratory to collect blood samples for an in vitro chemiluminescent immunoassay to quantify spike-protein IgG SARS-CoV-2 antibody titers developed 2 weeks, 3 months, and 6 months after completing vaccination. The first timing for antibody measurement was decided based on clinical trials' results for different COVID-19 vaccines, most of which had shown the greatest antibody titers 2 weeks after completing vaccination [21–24]. Antibody titers were reported in the WHO international standard binding antibody units (BAU)/mL [25]. The blood samples were destroyed after the laboratory result was released. In every timepoint, participants were also asked about the development of symptomatic SARS-CoV-2 infection and its severity, namely, the need for hospitalization and admission to an intensive care unit.

Our secondary aim was to assess differences between groups regarding seronegative responses, suboptimal antibody titers, and SARS-CoV-2 infection, using subgroup analysis considering their age, gender, cause and severity of cirrhosis, use of immunosuppressant drugs, relevant comorbidities, and type of vaccine given. To compare patients in terms of humoral response, two cutoffs for spike-protein SARS-CoV-2 IgG antibody titers were defined: titers under 33.8 BAU/mL were classified as a seronegative result (the 33.8 BAU/mL was the threshold for positivity, as determined by the laboratory performing the antibody measurements [*Centro de Medicina Laboratorial Germano de Sousa*]), and titers under 200 BAU/mL were subjectively considered suboptimal levels (defined upon results from one of the first studies of vaccine immunogenicity in CLD patients [26]).

Table 1. Patients' baseline characteristics

Characteristic	Total (*n* = 124)
Male gender, *n* (%)	100 (81)
Age, years, mean±SD [min–max]	61±10 [32–85]
Cause of LC (alone or in combination), *n* (%)	
Alcohol	76 (61)
Hepatitis C infection	36 (29)
Metabolic associated fatty liver disease	14 (11)
Hepatitis B infection	9 (7)
AIH	9 (7)
Other	8 (6)
Use of immunosuppressant drugs, *n* (%)	9 (7)
Features of portal hypertension, *n* (%)	85 (69)
Previous cirrhosis decompensation, *n* (%)	52 (42)
Ascites	42 (34)
Portal hypertensive bleeding	17 (14)
Hepatic encephalopathy	10 (8)
Spontaneous bacterial peritonitis	5 (4)
Hepatorenal syndrome	2 (2)
Child-Pugh score, *n* (%)	
A	98 (79)
B	23 (19)
C	3 (2)
MELD-Na score, mean±SD	11±4
Hepatocellular carcinoma, *n* (%)	8 (6)
Portal vein thrombosis, *n* (%)	3 (2)
Other relevant comorbidities, *n* (%)	
None	77 (62)
Hypertension	21 (17)
Diabetes	19 (15)
Obesity	8 (6)
Dyslipidemia	8 (6)
Cardiac disease	6 (5)

Max, maximum; min, minimum; no., number; SD, standard deviation.

Statistical Analysis

Statistical analysis was performed using Stata®17 (StataCorp, College Station, TX, USA). Continuous variables were described by their mean ± standard deviation (SD) or median and interquartile range [Q1; Q3], according to the observed distribution of the variables (normal or other, respectively), along with their minimum (min) and maximum (max) values when appropriate. Categorical variables were presented by observed absolute (*n*) and relative (%) frequencies. The comparison of antibody titers between two groups was made using Student's *t* test for independent samples for normally distributed data

and the Mann-Whitney U test for data without normal distribution. The association between SARS-CoV-2 infection and qualitative variables was assessed using the χ^2 test. Group differences in terms of seronegative, suboptimal antibody responses, and SARS-CoV-2 infection were described and assessed in a multivariable logistic regression model through a stepwise approach to fit the model and find independent clinical predictors at baseline considering the patients' characteristics and type of vaccine administrated, with reported odds ratios and 95% confidence intervals whenever appropriate. A p value <0.05 was considered statistically significant.

Results

We included 124 patients in the study, 81% males, mean aged 61 ± 10 years. Alcohol was the most common cause of LC (61%). Nine (7%) patients were under immunosuppressant drugs for AIH. Sixty-nine percent of the patients had features of portal hypertension, 42% had a previous cirrhosis decompensation, and 21% had a CPT score of B/C. Table 1 outlines patients' baseline characteristics.

All the patients were fully vaccinated between May and August 2021. The type of vaccine administrated was one of the four approved in Portugal at that time: Pfizer/Comirnaty® [BNT162b2] (n = 59, 48%), AstraZeneca/Vaxzevria® [ChAdOx1nCoV-19] (n = 45, 36%), Moderna/Spikevax® [mRNA-1273] (n = 14, 11%), and Janssen/Jcovden® [Ad26.COV2.S] (n = 6, 5%). They were followed during a mean period of 221 ± 26 days, until the timing of the booster dose administration, SARS-CoV-2 infection, or death (shown in Fig. 1). The 6-month analysis excluded 67 patients who had an earlier booster dose, 7 who were infected with SARS-CoV-2, and 2 who died of unrelated causes to SARS-CoV-2 infection. During follow-up, there was no progression or newly-onset complications associated with the patients' underlying liver condition.

Eighteen percent of the patients (n = 22) reported vaccine-related adverse events, none of them serious. These complaints included fever (6%), myalgia (6%), fatigue (5%), diarrhea (2%), and headache (1%). They were all self-limited and did not make them seek medical care.

As for humoral response, the median spike-protein IgG SARS-CoV-2 antibody titers were 1,185 [280; 2,080] BAU/mL at 2 weeks, 301 [72; 1,175] BAU/mL at 3 months, and 192 [49; 656] BAU/mL at 6 months after completing vaccination (shown in Fig. 2). We reported negative (<33.8 BAU/mL) and suboptimal (<200 BAU/mL) antibody titers in 8% and 23% of the patients at 2 weeks, 16% and 38% at 3 months, and 22% and 48% at 6 months.

Older age was associated with negative and suboptimal antibody titers in almost every timepoint analyzed using a multivariable logistic regression model (Table 2). Patients with negative or suboptimal titers at 2 weeks were 65 ± 10 years compared to 60 ± 10 years (p = 0.049) and 64 ± 11 years versus 59 ± 10 years (p = 0.032), respectively. Similar differences were also seen at 3 months, when patients with negative or suboptimal titers were 66 ± 9 years versus 60 ± 10 years (p = 0.013) and 65 ± 8 versus 59 ± 10 years (p = 0.002), respectively, and at 6 months, with negative or suboptimal titers in patients aged 68 ± 6 years compared to 55 ± 8 years (p = 0.050) and 60 ± 9 years versus 54 ± 7 years (p = 0.084).

Alcoholic LC was associated with suboptimal antibody titers at 2 weeks (29% compared to 14% in nonalcoholic cirrhosis, p = 0.032). Considering the 9 patients with AIH under immunosuppressive drugs, 11% had negative and 22% had suboptimal antibody levels at both 2 weeks and 3 months after completing vaccination, without statistically significant differences compared to the remaining patients; only 2 patients were eligible for the 6-month analysis, and this was therefore not performed. Group differences regarding liver disease severity and antibody titers are shown in Figure 3 and were not statistically significant.

The mRNA-1273 vaccine had the highest median antibody titers throughout every timepoint of analysis, followed by the BNT162b2, as shown in Figure 4. Adenovirus vector vaccines (Ad26.COV2.S and ChAdOx1nCoV-19) were associated with lower median antibody titers when compared to mRNA vaccines, both at 2 weeks and 3 months (shown in Fig. 4; Table 2). In the 6-month analysis, there was a lower number of patients in each group; therefore, these differences were not analyzed.

Eleven patients (9%) developed SARS-CoV-2 infection during the follow-up period, occurring 3.8–6.6 months after completing vaccination. All of them had asymptomatic (n = 1) or mild (n = 10) disease, without the need for hospitalization. They were 73% males, with a mean age of 60 ± 11 years. Except for alcoholic LC, which was associated with lower rates of SARS-CoV-2 infection (p = 0.005), we did not find other associations between patients' characteristics and the development of SARS-CoV-2 infection (Table 3), namely, type of vaccine administrated and spike-protein SARS-CoV-2 IgG antibody titers. In fact, 8 patients (73%) had spike-protein SARS-CoV-2 IgG antibody titers greater than 200 BAU/mL on the 3-month analysis.

Discussion

Infections are a common cause of decompensation and death in patients with LC. Their dysregulated immune system makes them not only more susceptible to certain

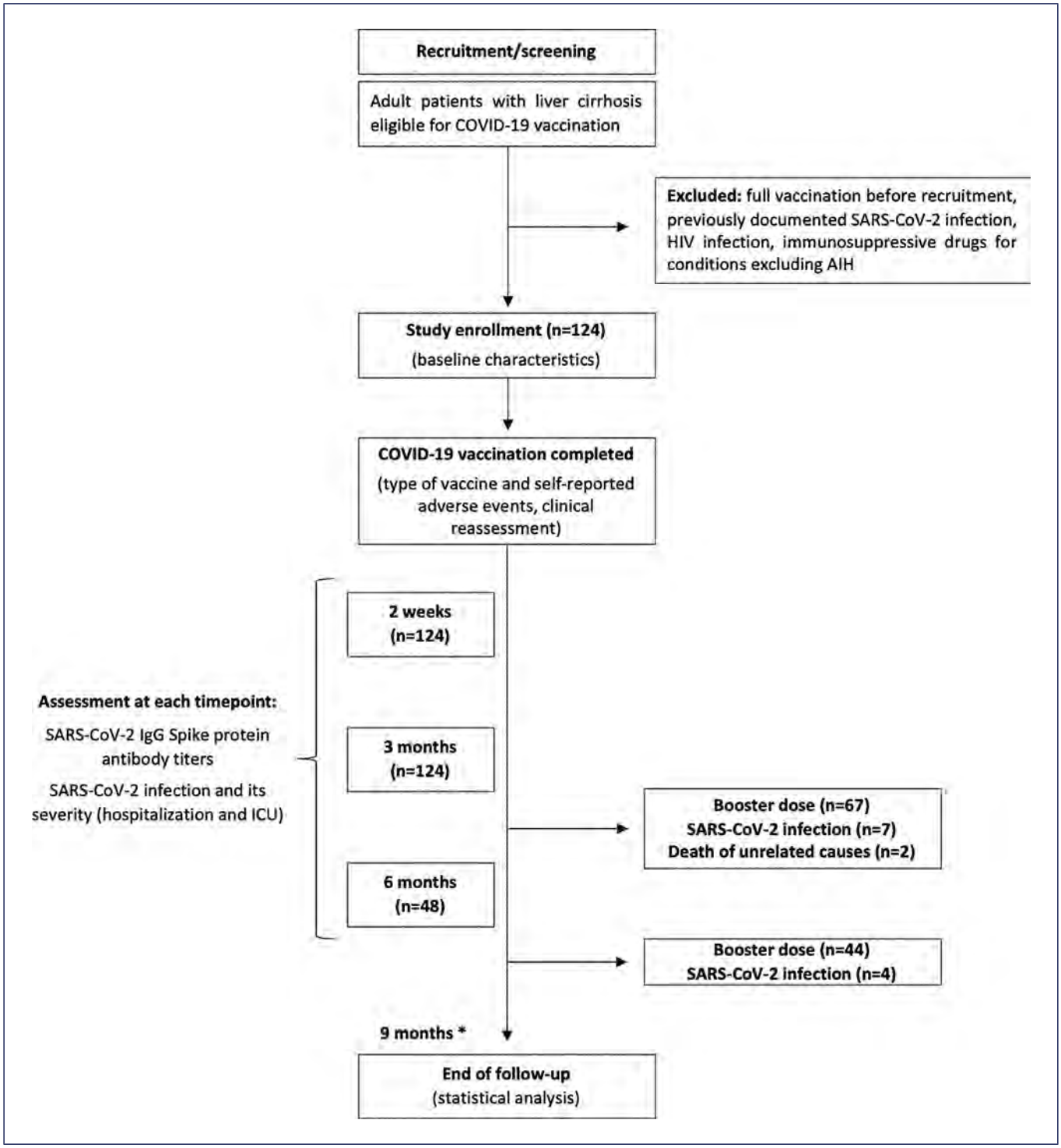


Fig. 1. Flowchart of patient enrollment and follow-up. *The end of follow-up by 9 months was related to the timing of the last booster dose given. AIH, autoimmune hepatitis; HIV, human immunodeficiency virus; ICU, intensive care unit; IgG, immunoglobulin G.

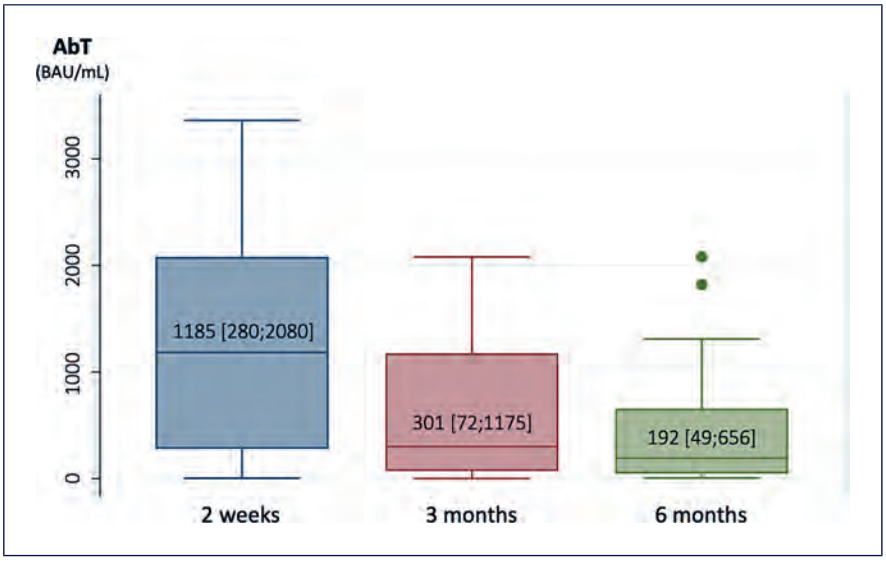


Fig. 2. Median [Q1; Q3] spike-protein IgG SARS-CoV-2 antibody titers (AbT) at 2 weeks, 3 months, and 6 months after completing vaccination.

Table 2. Multivariable logistic regression analysis of patients' characteristics and positive (>33.8 BAU/mL) or optimal (>200 BAU/mL) antibody titers at 2 weeks and 3 months

	Positive antibody titers			Optimal antibody titers		
	OR	95% CI	*p* value	OR	95% CI	*p* value
Characteristic: 2 weeks						
Age (years)	0.902	0.814–0.998	**0.049**	0.934	0.878–0.994	**0.032**
Male gender	0.650	0.081–5.203	0.685	0.618	0.135–2.814	0.534
Alcoholic LC	0.131	0.014–1.256	0.078	0.197	0.045–0.869	**0.032**
Comorbidities within metabolic syndrome and/or cardiovascular disease	0.610	0.088–4.189	0.615	0.697	0.215–2.265	0.549
Use of immunosuppressant drugs	0.228	0.015–3.389	0.283	0.409	0.051–3.309	0.402
Features of portal hypertension	1.600	0.403–6.357	0.504	2.263	0.624–8.208	0.214
Previous cirrhosis decompensation	1.355	0.320–5.739	0.679	2.149	0.619–7.460	0.228
Child-Pugh score B/C	0.886	0.170–4.597	0.886	1.549	0.325–7.382	0.583
Type of vaccine	0.131	0.034–0.513	**0.003**	0.222	0.104–0.473	<**0.001**
Characteristic: 3 months						
Age (years)	0.886	0.806–0.974	**0.013**	0.906	0.852–0.964	**0.002**
Male gender	0.179	0.030–1.049	0.057	0.864	0.207–3.596	0.840
Alcoholic LC	0.226	0.036–1.399	0.110	0.321	0.091–1.131	0.077
Comorbidities within metabolic syndrome and/or cardiovascular disease	0.571	0.131–2.484	0.455	1.447	0.485–4.316	0.507
Use of immunosuppressant drugs	0.398	0.027–5.939	0.504	0.876	0.106–7.243	0.903
Features of portal hypertension	1.133	0.367–3.498	0.828	1.627	0.533–4.962	0.392
Previous cirrhosis decompensation	0.709	0.153–3.285	0.661	2.861	0.862–9.499	0.086
Child-Pugh score B/C	1.600	0.327–7.815	0.561	1.433	0.322–6.370	0.636
Type of vaccine	0.155	0.049–0.483	**0.001**	0.331	0.175–0.625	**0.001**

OR, odds ratio; 95% CI, 95% confidence interval.

infections but also more likely to develop serious disease [27]. In a large international cohort of unvaccinated CLD patients, SARS-CoV-2 infection in patients with LC led to liver decompensation in 46% of the cases, half of them with acute-on-chronic liver failure, and a 32% mortality rate, compared to 8% in patients without cirrhosis ($p < 0.001$) [12].

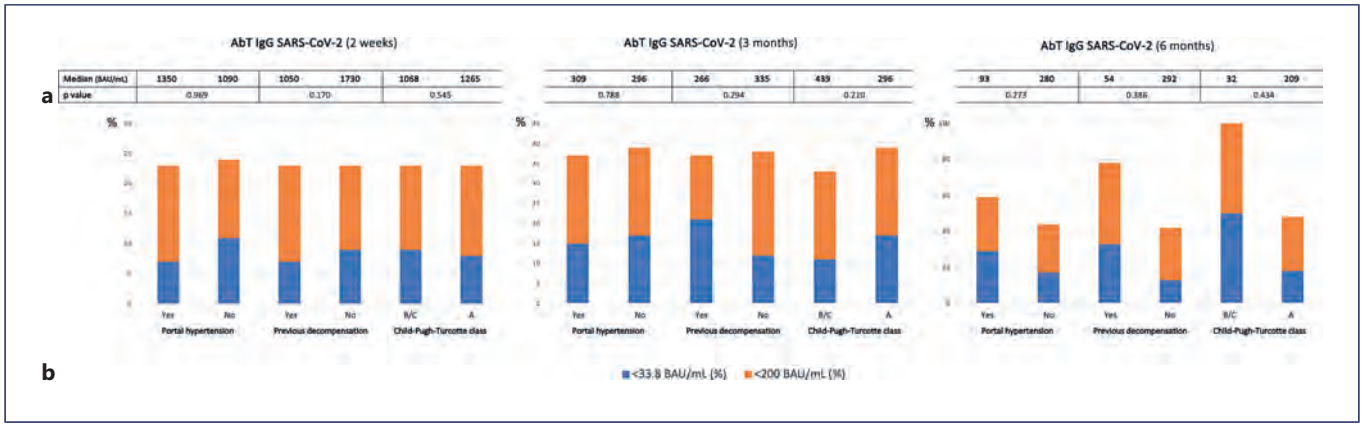


Fig. 3. Group comparisons in terms of liver disease severity regarding spike-protein IgG SARS-CoV-2 antibody titers (AbT) at 2 weeks, 3 months, and 6 months after completing vaccination. **a** Median AbT (BAU/mL) in each group; *p* values were obtained using a Mann-Whitney U test. **b** Proportion (%) of patients in each group with AbT less than 33.8 BAU/mL (blue) and 200 BAU/mL (orange).

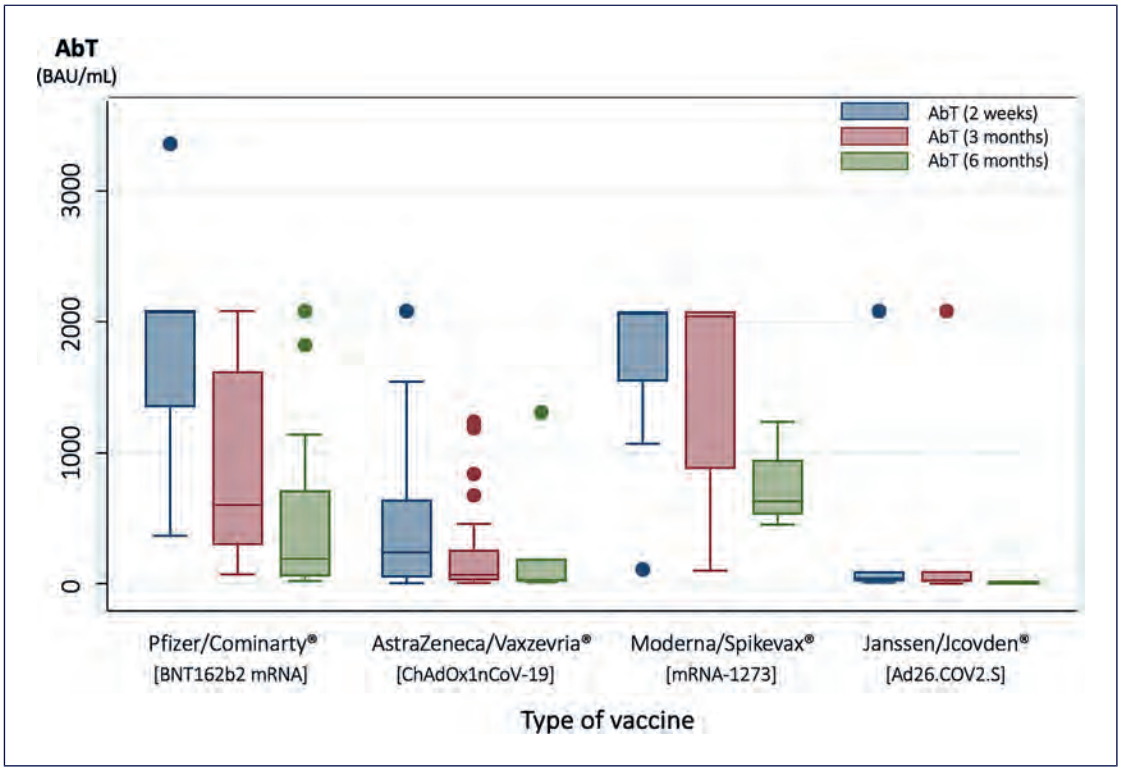


Fig. 4. Median spike-protein IgG SARS-CoV-2 antibody titers (AbT) according to type of vaccine administrated at 2 weeks, 3 months, and 6 months after completing vaccination.

Although nowadays much evidence regarding COVID-19 vaccination in LC patients has been published, most of the studies were based on retrospective cohorts from large databases [14, 17, 28] and did not simultaneously assess safety, humoral, and clinical responses in patients with LC.

Table 3. Group comparisons regarding the development of SARS-CoV-2 infection

Characteristic	No infection (*n* = 113)	SARS-CoV-2 infection (*n* = 11)	*p* value
Male gender, *n* (%)	92 (81)	8 (73)	0.312
Age, years, mean±SD	61±11	60±11	0.889
Cause of LC (alone or in combination), *n* (%)			
Alcohol	74 (65)	2 (18)	**0.005**
Hepatitis C infection	32 (28)	4 (36)	0.459
Metabolic associated fatty liver disease	14 (12)	0 (0)	0.232
Hepatitis B infection	8 (7)	1 (9)	0.747
AIH under immunosuppressants	7 (6)	2 (18)	0.114
Features of portal hypertension, *n* (%)	78 (69)	7 (63)	0.541
Previous cirrhosis decompensation, *n* (%)	47 (42)	5 (45)	0.929
Child-Pugh score B/C, *n* (%)	23 (20)	3 (27)	0.989
MELD-Na score, mean±SD	11±4	11±3	0.959
Hepatocellular carcinoma, *n* (%)	8 (6)	0 (0)	0.368
Comorbidities within metabolic syndrome and/or cardiovascular disease, *n* (%)	44 (39)	3 (27)	0.284
Type of vaccine, *n* (%)			
BNT162b2	52 (46)	7 (63)	0.865
ChAdOx1nCoV-19	43 (38)	2 (18)	
mRNA-1273	13 (12)	1 (9)	
Ad26.COV2.S	5 (4)	1 (9)	
Antibody titers, BAU/mL, median [Q1–Q3]			
2 weeks	1,185 [268–2,080]	1,540 [403–2,020]	0.868
3 months	301 [70–1,240]	344 [228–808]	0.566

Max, maximum; min, minimum; no., number; Q1, first quartile; Q3, third quartile; SD, standard deviation.

In our study, side effects were observed in 18% of the patients, but they were all mild and consisted mostly of fever, myalgia, and fatigue, similar to the results reported from clinical trials [29]. The good safety profile of COVID-19 vaccines in LC was in line with other studies in this population [14]. A safety assessment of the ChAdOx1-nCOV vaccine [30] showed a rate of systemic adverse events of 22%, with fever being the most common symptom, and all of them were mild and transient, just like in other cohorts of patients [31].

Specific concerns of safety of COVID-19 vaccination in this population would be vaccine-triggered immune-mediated hepatitis and vaccine-induced thrombotic thrombocytopenia, resulting in splanchnic and hepatosplenic thrombosis [11]. However, immune-mediated hepatitis has been rarely reported, and no causal link to the vaccine has yet been established, to the point that the occurrence of liver injury after vaccination is not a contraindication to subsequent vaccination. Thrombotic thrombocytopenia, a rare event after COVID-19 vaccination with adenoviral vector vaccines which could lead to cirrhosis decompensation, was also not observed in our study, even though we acknowledge that our sample size was too low to detect these infrequent side effects.

In this study, we assessed immunological response through humoral response with spike-protein IgG SARS-CoV-2 antibody titer measurement at three different timepoints.

We observed a seroconversion rate of 92% in LC patients 2 weeks after vaccination. A meta-analysis on COVID-19 vaccine immunogenicity among CLD patients [14] reported a good humoral response to inactivated and mRNA COVID-19 vaccines, with total seroconversion rates of 85% either in cirrhotic or non-cirrhotic CLD patients (with pooled seroconversion rates of neutralizing and anti-spike antibodies of 84% and 92%, respectively). However, even though patients with LC seem to develop similar seroconversion rates, some studies have described lower antibody titers compared to non-cirrhotics. For example, a study by Willuweit et al. [32] reported similar seroconversion rates (96% vs. 99%, $p = 0.400$) after 2 doses of the BNT162b2 vaccine but lower IgG SARS-CoV-2 titers (939 vs. 1,905 BAU/mL, $p = 0.0001$) in patients with LC compared to controls. Importantly, the timing for antibody measurement was

different between groups (median 69 vs. 56 days for cirrhotics and controls, respectively), which may have affected the results. Another study in patients vaccinated with BNT162b2 (70.3%), mRNA-1273 (18.9%), or ChAdOx1nCoV-19 (10.8%) [15] reported a nonsignificant trend to lower IgG antibody levels in patients with cirrhosis or advanced liver fibrosis (F3–F4) compared with healthy controls, 2 weeks and 6 months after vaccination.

Antibody titers decreased in subsequent evaluations during our follow-up period. This waning humoral response has been described in a study by Levin et al. [33] in healthcare workers, with the level of IgG antibodies decreasing at a consistent rate up to 6 months of follow-up after the second dose of the BNT162b2 vaccine. In a study comparing LC patients with controls, this waning was seen similarly in both groups at 6 months [15].

We did not evaluate T-cell reactivity. A decreased T-cell response could be a possible explanation for the occurrence of SARS-CoV-2 infection despite adequate antibody levels. In fact, patients with SARS-CoV-2 infection had nonsignificant greater median antibody titers than uninfected patients, which support the role of other immune mechanisms in conferring protection. A greater cellular response could explain lower rates of infection in subgroups with suboptimal antibody titers as reported for alcoholic LC. Previously published data have been inconsistent, with studies reporting T-cell responses in cirrhotic patients similar to controls [16] and others showing an impairment in T-cell reactivity in cirrhotic patients compared to controls (36% vs. 6%) [34]. These studies are limited by the different timing for antibodies' measurement, which may have affected the results.

In our cohort of vaccinated LC patients, only 9% (ranging from 4% to 17% according to the type of vaccine) developed COVID-19 during the peak of the pandemic. None of the patients developed moderate-severe SARS-CoV-2 infection, and no hospital admission, intensive care unit admission, or death were reported. We acknowledge that the participation in this study could have led to a subject bias with potential overestimation of the clinical efficacy, with patients avoiding high-risk behaviors that would increase the likelihood of being infected with SARS-CoV-2. However, our results are in line with the ones reported in the literature, either in healthy or in CLD subjects. Indeed, clinical trials have reported a vaccine efficacy against symptomatic/moderate/severe COVID-19 of 95%, 94%, 70%, and 67% for the BNT162b2, mRNA-1273, ChAdOx1nCoV-19, and Ad26.COV2.S vaccines, respectively [35].

Similar good results were also described in previous retrospective studies in CLD [28]. A study from a large US database on 20,037 liver cirrhotic patients propensity-matched to 20,037 non-cirrhotic controls vaccinated with at least one mRNA vaccine dose [18] showed a delayed, but robust 78.6% reduction on the incidence of COVID-19 infection after the second dose, and an excellent reduction of 100% in COVID-19-related hospitalization and mortality in patients with LC. Another large cohort of 68,048 unvaccinated compared to 10,441 vaccinated CLD patients with cirrhosis [17] reported that 15% versus 3.7% developed SARS-CoV-2 infection and 15.2% versus 7.7% of these needed mechanical ventilation or died in a 30-day follow-up, respectively.

We found no correlation between antibody titers and the development of SARS-CoV-2 infection in our population, with 73% of the patients that developed SARS-CoV-2 infection presenting adequate titers 3 months after vaccination. A systematic review on the correlation of antibody levels to vaccine efficacy [36] reported that, while in some studies there was a correlation between them, in others there was an inverse relationship between antibody levels and infection incidence, risk, or viral load, suggesting that both humoral immunity and other immune components contribute to protection.

In our study, older age was associated with lower antibody titers, which has been described in previous studies either in patients with CLD [26] or in healthcare workers [33]. These studies also reported male gender and immunosuppression as risk factors for worse humoral responses [26, 33]. In our population, we did not see these associations, which may be explained by the small proportion of females and patients under immunosuppression limiting adequate group comparisons.

Despite not finding statistically significant associations, limited by the small group size, from the 9 patients under immunosuppressants, 2 (18%) developed SARS-CoV-2 infection, compared to 9 (8%) of the remaining participants. This suggests that the first may have been more susceptible to lower vaccine efficacy, although infection severity was not worse. Previous studies have reported a negative correlation between immunosuppressive treatment and anti-SARS-CoV-2 antibody titers and neutralizing activity compared to other patients with CLD [37] and a lower T-cell response of patients with AIH when compared to patients with primary biliary cholangitis or primary sclerosing cholangitis [38], even in the ones without immunosuppressive treatment. Despite these findings, clinical efficacy of SARS-CoV-2 did not seem impaired, with reports of a significant reduction on the risk of COVID-19 severity and mortality in patients with AIH [39].

In our cohort, patients with alcoholic LC were associated with suboptimal antibody responses at 3 months but a lower SARS-CoV-2 infection rate. A possible explanation would be a greater influence of the unmeasured T-cell response in preventing SARS-CoV-2 infection, as previously mentioned. Besides, our inability to assess patients' lifestyle behaviors that could make them more susceptible to SARS-CoV-2 infection, despite adequate immunization, should be taken into account to explain these differences.

In our study, we did not find differences in antibody titers of patients with more advanced liver disease, when compared to patients without previous decompensations, without portal hypertension or with CPT A, except for a nonsignificant trend to lower titers by 6 months. Although the small proportion of patients with CPT scores B and C compared with CPT A (21% vs. 79%) could have impacted these results, the effect of the stage of LC is controversial in the literature. Some studies have reported that patients with decompensated cirrhosis demonstrated suboptimal humoral (measured through anti-spike antibodies) and cellular immune responses against recombinant and inactivated COVID-19 vaccines [40] and that CPT B/C cirrhosis was an independent risk factor for negative neutralizing antibodies [41]. However, other studies reported no differences in antibody responses regarding compensated versus decompensated cirrhosis [30, 31] or according to MELD and CPT scores [32].

In our results, adenovirus vector vaccines were associated with lower antibody titers, compared to mRNA vaccines. These differences have been previously reported in the literature. In a meta-analysis on COVID-19 vaccine immunogenicity among CLD patients, including four studies on inactivated vaccines and three on mRNA vaccines, seroconversion rates were 86% in inactivated and 89% in mRNA vaccines [14]. In a study on CLD patients, the type of vaccine was associated with different humoral responses, which were lower for ChAdOx1-nCoV-19, followed by BNT162b2 mRNA and finally mRNA-1273, although these did not appear to associate with clinical efficacy [15]. Collier et al. [42] reported that mRNA-1273 vaccines provided initial high peak antibody responses that declined sharply by 6 months, whereas Ad26.COV2.S vaccines induced lower initial antibody responses which were relatively stable over time, and these differences in humoral kinetics could explain equivalent clinical responses. Similarly, in our study, these humoral differences were not related to lower clinical efficacy. A large case-control study in LC reported that differences between groups regarding vaccine clinical efficacy between Ad.26.COV2.S and mRNA vaccines were also not statistically significant [43].

An important limitation of this study was the fast vaccination rate and the prompt introduction of a booster dose in Portugal, which led to the exclusion of many patients who were already vaccinated at the time of recruitment and earlier termination of follow-up of the ones vaccinated with an additional dose. Also, we did not have a control group, and comparisons were made with available data from the general population from clinical trials and people included in real-life studies. Another limitation was the absence of an index antibody titer assessment before vaccination and, therefore, the impossibility to ascertain previously unknown asymptomatic SARS-CoV-2 infection before vaccination, which would affect humoral and clinical outcomes. Finally, our immunological assessment only included the humoral response but not cellular responses to the vaccine, which are also known to play a role in the development of immunity against the virus.

The main strengths of this study are (1) its prospective nature with close follow-up of all the patients since enrollment during a median period of 7 months (whereas most of the literature available refers to retrospective cohorts); (2) the assessment of both humoral and clinical outcomes in every patient throughout time. We used the same standardized techniques for antibody titer measurement within one laboratory group during different well-specified timepoints in every patient. Furthermore, all the patients were monitored frequently for clinical endpoints regarding safety and efficacy, providing an adequate and detailed evaluation of this susceptible population.

In conclusion, COVID-19 vaccines in patients with LC were safe, resulting in no serious adverse events. The humoral and clinical responses were good, even when compared to results reported for the general population in trials and healthy controls in real-life studies. We did not find an association between humoral and clinical responses, suggesting that further vaccination boosters should not be decided based on antibody titers. The only associations with lower humoral responses were older age and adenovirus vector vaccines. The severity of liver disease did not have an impact on humoral responses, even though we did not evaluate cellular immune responses. These results highlight the important role of COVID-19 vaccination in this susceptible group of patients, as recommended by international guidelines and national policies, in order to prevent SARS-CoV-2 infection, a precipitating factor for cirrhosis decompensation and death.

Acknowledgments

We would like to thank *Centro de Medicina Laboratorial Germano de Sousa* for its collaboration with blood sample collection to quantify spike-protein IgG SARS-CoV-2 antibody titers in many different locations throughout the country.

Statement of Ethics

This study protocol was reviewed and approved by the Ethics Committees of the following hospitals: Centro Hospitalar Universitário de Lisboa Central (coordinating center – process number: 1080/2021), Centro Hospitalar de Leiria, Centro Hospitalar Universitário de Coimbra, Hospital de Vila Franca de Xira, Centro Hospitalar Lisboa Ocidental, and Hospital Beatriz Ângelo. All the participants enrolled in the study provided informed consent, in accordance with the tenets of the Declaration of Helsinki.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was funded by *Associação Portuguesa para o Estudo do Fígado*, which supported the costs of the antibody titer analysis of every patient at each timepoint.

Author Contributions

Maria Inês Canha and Filipe Calinas were involved in every step of the conception of the study protocol, in the analysis and interpretation of data, in drafting the manuscript and agreed to be accountable for all aspects of this work. Mário Jorge Silva, Maria Azevedo Silva, Mara Costa, Rita Saraiva, André Ruge, Mariana Machado, Catarina Félix, Bárbara Morão, Pedro Narra Figueiredo, Milena Mendes, and Carina Leal made substantial contributions to the acquisition of data for the work, revised the manuscript critically for important intellectual content, approved its final version, and agreed to be accountable for its aspects.

Data Availability Statement

Mostly all data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. N Engl J Med. 2020;382(8):727–33.
2 Bettini E, Locci M. SARS-CoV-2 mRNA vaccines: immunological mechanism and beyond. Vaccines. 2021;9(2):147–20.
3 Sahin U, Muik A, Derhovanessian E, Vogler I, Kranz LM, Vormehr M, et al. COVID-19 vaccine BNT162b1 elicits human antibody and TH1 T cell responses. Nature. 2020;586(7830):594–9.
4 Jin P, Li J, Pan H, Wu Y, Zhu F. Immunological surrogate endpoints of COVID-2019 vaccines: the evidence we have versus the evidence we need. Signal Transduct Target Ther. 2021;6(1):48–7.
5 Grigoryan L, Pulendran B. The immunology of SARS-CoV-2 infections and vaccines. Semin Immunol. 2020;50:101422.
6 Mehrotra DV, Janes HE, Fleming TR, Annunziato PW, Neuzil KM, Carpp LN, et al. Clinical endpoints for evaluating efficacy in COVID-19 vaccine trials. Ann Intern Med. 2021;174(2):221–8.
7 Marjot T, Webb GJ, Barritt AS, Ginès P, Lohse AW, Moon AM, et al. SARS-CoV-2 vaccination in patients with liver disease: responding to the next big question. Lancet Gastroenterol Hepatol. 2021;6(3):156–8.
8 Janssen Science. Use of janssen COVID-19 vaccine in patients with hepatic impairment; 2023. Available from: https://www.janssenscience.com/products/janssen-covid-19-vaccine/medical-content/use-of-janssen-covid-19-vaccine-in-patients-with-hepatic-impairment [Accessed on 28 04 2023].
9 Fix OK, Blumberg EA, Chang K, Chu J, Chung RT, Goacher EK, et al. American association for the study of liver diseases expert panel consensus statement: vaccines to prevent coronavirus disease 2019 infection in patients with liver disease. Hepatology. 2021;74(2):1049–64.
10 Cornberg M, Buti M, Eberhardt CS, Grossi PA, Shouval D. EASL position paper on the use of COVID-19 vaccines in patients with chronic liver diseases, hepatobiliary cancer and liver transplant recipients. J Hepatol. 2021;74(4):944–51.
11 Marjot T, Eberhardt CS, Boettler T, Belli LS, Berenguer M, Buti M, et al. Impact of COVID-19 on the liver and on the care of patients with chronic liver disease, hepatobiliary cancer, and liver transplantation: an updated EASL position paper. J Hepatol. 2022;77(4):1161–97.
12 Marjot T, Moon AM, Cook JA, Abd-elsalam S, Aloman C, Armstrong MJ, et al. Outcomes following SARS-CoV-2 infection in patients with chronic liver disease: an international registry study. J Hepatol. 2021;74(3):567–77.
13 Bajaj JS, Garcia-Tsao G, Biggins SW, Kamath PS, Wong F, McGeorge S, et al. Comparison of mortality risk in patients with cirrhosis and COVID-19 compared with patients with cirrhosis alone and COVID-19 alone: multicentre matched cohort. Gut. 2021;70(3):531–6.
14 Cheung K, Mok C, Mao X, Zhang R, Hung I, Seto W, et al. COVID-19 vaccine immunogenicity among chronic liver disease patients and liver transplant recipients: a meta-analysis. Clin Mol Hepatol. 2022;28(4):890–911.
15 Simão AL, Palma CS, Izquierdo-Sanchez L, Putignano A, Carvalho-Gomes A, Posch A, et al. Cirrhosis is associated with lower serological responses to COVID-19 vaccines in patients with chronic liver disease. JHEP Rep. 2023;5(5):100697.
16 Iavarone M, Tosetti G, Facchetti F, Topa M, Er JM, Hang SK, et al. Spike-specific humoral and cellular immune responses after COVID-19 mRNA vaccination in patients with cirrhosis: a prospective single center study. Dig Liver Dis. 2023;55(2):160–8.
17 Ge J, Digitale JC, Pletcher MJ, Lai JC; N3C Consortium. Breakthrough SARS-CoV-2 infection outcomes in vaccinated patients with chronic liver disease and cirrhosis: a National COVID Cohort Collaborative study. Hepatology. 2023;77(3):834–50.
18 John BV, Deng Y, Scheinberg A, Mahmud N, Taddei TH, Kaplan D, et al. Association of BNT162b2 mRNA and mRNA-1273 vaccines with COVID-19 infection and hospitalization among patients with cirrhosis. JAMA Intern Med. 2021;181(10):1306–14.

19 Bahrs C, Harrison N. Vaccine response in the immunocompromised patient with focus on cellular immunity. Vaccines. 2022;10(6):882–3.
20 World Health Organization. Living Guidance for clinical management of COVID-19: World Health Organization; 2021. Available from: https://www.who.int/publications/i/item/WHO-2019-nCoV-clinical-2021-1 [Accessed on 08 09 2023].
21 Mulligan MJ, Lyke KE, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Phase I/II study of COVID-19 RNA vaccine BNT162b1 in adults. Nature. 2020;586(7830):589–93.
22 Walsh EE, Frenck RW Jr, Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and immunogenicity of two RNA-based covid-19 vaccine candidates. N Engl J Med. 2020; 383(25):2439–50.
23 Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. Lancet. 2020; 396(10249):467–78.
24 Widge A, Rouphael N, Jackson L, Anderson E, Roberts P, Makhene M, et al. Durability of responses after SARS-CoV-2 mRNA-1273 vaccination. N Engl J Med. 2021;384(1):80–2.
25 Kristiansen PA, Page M, Bernasconi V, Mattiuzzo G, Dull P, Makar K, et al. WHO International Standard for anti-SARS-CoV-2 immunoglobulin. Lancet. 2021;397(10282): 1347–8.
26 Shafrir A, Amer J, Hakimian D, Milgrom Y, Massarwa M, Hazou W, et al. Advanced liver fibrosis correlates with impaired efficacy of pfizer-BioNTech COVID-19 vaccine in medical employees. Hepatol Commun. 2022; 6(6):1278–88.
27 Ekpanyapong S, Reddy KR. Infections in cirrhosis. Curr Treat Options Gastroenterol. 2019;17(2):254–70.
28 Beran A, Mhanna A, Mhanna M, Hassouneh R, Abuhelwa Z, Mohamed MFH, et al. Real-world effectiveness of COVID-19 vaccination in liver cirrhosis: a systematic review with meta-analysis of 51,834 patients. Proc. 2023; 36(2):151–6.
29 Kaur RJ, Dutta S, Bhardwaj P, Charan J, Dhingra S, Mitra P, et al. Adverse events reported from COVID-19 vaccine trials: a systematic review. Indian J Clin Biochem. 2021;36(4):427–39.
30 Singh A, De A, Singh MP, Rathi S, Verma N, Premkumar M, et al. Antibody response and safety of ChAdOx1-nCOV (covishield) in patients with cirrhosis: a cross-sectional, observational study. Dig Dis Sci. 2023;68(2): 676–84.
31 Ai J, Wang J, Liu D, Xiang H, Guo Y, Lv J, et al. Safety and immunogenicity of SARS-CoV-2 vaccines in patients with chronic liver diseases (CHESS-NMCID 2101). A Multicenter Study. 2022;7(20):1516–24.
32 Willuweit K, Frey A, Passenberg M, Korth J, Saka N, Anastasiou OE, et al. Patients with liver cirrhosis show high immunogenicity upon COVID-19 vaccination but develop premature deterioration of antibody titers. Vaccines. 2022;10(3):377–9.
33 Levin EG, Lustig Y, Cohen C, Fluss R, Indenbaum V, Amit S, et al. Waning immune humoral response to BNT162b2 covid-19 vaccine over 6 months. N Engl J Med. 2021;385(24):e84.
34 Al-Dury S, Waern J, Waldenström J, Alavanja M, Saed HH, Törnell A, et al. Impaired SARS-CoV-2-specific T-cell reactivity in patients with cirrhosis following mRNA COVID-19 vaccination. JHEP Rep. 2022; 4(7):100496.
35 Angeli F, Spanevello A, Reboldi G, Visca D, Verdecchia P. SARS-CoV-2 vaccines: lights and shadows. Eur J Intern Med. 2021; 88(4):1–8.
36 Perry J, Osman S, Wright J, Richard-Greenblatt M, Buchan SA, Sadarangani M, et al. Does a humoral correlate of protection exist for SARS-CoV-2? A systematic review. PLoS One. 2022;17(4):e0266852–20.
37 Bakasis AD, Bitzogli K, Mouziouras D, Pouliakis A, Roumpoutsou M, Goules AV, et al. Antibody responses after SARS-CoV-2 vaccination in patients with liver diseases. Viruses. 2022;14(2):207–9.
38 Duengelhoef P, Hartl J, Rüther D, Steinmann S, Brehm TT, Weltzsch JP, et al. SARS-CoV-2 vaccination response in patients with autoimmune hepatitis and autoimmune cholestatic liver disease. United Eur Gastroenterol J. 2022;10(3):319–29.
39 Efe C, Taşçılar K, Gerussi A, Bolis F, Lammert C, Ebik B, et al. SARS-CoV-2 vaccination and risk of severe COVID-19 outcomes in patients with autoimmune hepatitis. J Autoimmun. 2022;132(10):102906.
40 Kulkarni AV, Jaggaiahgari S, Iyengar S, Simhadri V, Gujjarlapudi D, Rugwani H, et al. Poor immune response to coronavirus disease vaccines in decompensated cirrhosis patients and liver transplant recipients. Vaccine. 2022;40(48):6971–8.
41 Wang J, Zhang Q, Ai J, Liu D, Liu C, Xiang H, et al. Safety and immunogenicity of SARS-CoV-2 vaccines in Chinese patients with cirrhosis: a prospective multicenter study. Hepatol Int. 2022;16(3):691–701.
42 Collier A, Yu J, McMahan K, Liu J, Chandrashekar A, Maron JS, et al. Differential kinetics of immune responses elicited by covid-19 vaccines. N Engl J Med. 2021; 385(21):2010–2.
43 John BV, Sidney Barritt A, Moon A, Taddei TH, Kaplan DE, Dahman B, et al. Effectiveness of COVID-19 viral vector Ad.26.-COV2.S vaccine and comparison with mRNA vaccines in cirrhosis. Clin Gastroenterol Hepatol. 2022;20(10):2405–8.e3.

GE – Portuguese Journal of Gastroenterology

Research Article

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Received: June 20, 2023
Accepted: November 23, 2023
Published online: January 29, 2024

Risk Factors in Serrated Pathway Lesions: N-Glycosylation Profile as a Potential Biomarker of Progression to Malignancy

Henrique Fernandes-Mendes[a, b] Catarina M. Azevedo[b, c] Mónica Garrido[d] Carolina Lemos[b, c] Isabel Pedroto[a, b] Salomé S. Pinho[c] Ricardo Marcos-Pinto[a, b] Ângela Fernandes[c]

[a]Department of Gastroenterology, Centro Hospitalar Universitário de Santo António, Porto, Portugal; [b]Instituto de Ciências Biomédicas Abel Salazar, University of Porto, Porto, Portugal; [c]Institute for Research and Innovation in Health (i3S), University of Porto, Porto, Portugal; [d]Department of Gastroenterology, Portuguese Oncology Institute of Porto (IPO Porto), Porto, Portugal

Keywords

Colorectal cancer · Serrated pathway · Serrated lesions · *N*-glycosylation · β1,6-GlcNAc branched *N*-glycans · Biomarker

Abstract

Introduction: The serrated pathway contributes to interval colorectal cancers, highlighting the need for new biomarkers to assess lesion progression risk. The β1,6-GlcNAc branched *N*-glycans expression in CRC cells was associated with an invasive phenotype and with immune evasion. Therefore, this study aims to identify potential risk factors for progression of serrated lesions (SLs) to malignancy, analyzing the *N*-glycosylation profile of epithelial/infiltrating immune cells. ***Methods:*** A retrospective cohort study was performed with data from 53 colonoscopies (48 patients). Sixty-three serrated pathway lesions (SPLs) were characterized based on *N*-glycosylation profile (lectin histochemistry/flow cytometry) and *MGAT5* expression. Statistical analysis was performed to search for associations between the glycoprofile and clinical variables from each patient. ***Results:*** Increased β1,6-GlcNAc branched *N*-glycans expression in epithelial cells is found associated with age ($p = 0.007$ in SPL), smoking ($p = 0.038$ in SL), increased BMI ($p = 0.036$ in sessile serrated lesions [SSL]), and polyp dimensions ≥10 mm ($p = 0.001$ in SL), while increased expression of these structures on immune cells is associated with synchronous CA number ($CD4^+$T cells: $p = 0.016$; $CD8^+$T cells: $p = 0.044$ in SL) and female gender ($p = 0.026$ in SL). Moreover, a lower high-mannose *N*-glycans expression in immune cells is associated with smoking ($p = 0.010$ in SPL) and synchronous CA presence ($p = 0.010$ in SPL). Higher expression of these glycans is associated with female ($p = 0.016$ in SL) and male ($p = 0.044$ in SL) gender, left colon location ($p = 0.028$), dysplasia ($p = 0.028$), and adenocarcinoma ($p = 0.010$). ***Conclusions:*** We identified an association between an abnormal glycoprofile and several clinical risk factors, proposing the *N*-glycosylation profile as a potential biomarker of tumor progression in the serrated pathway. The *N*-glycosylation anatomopathological profile analysis could be further used to decide shorter interval follow-up in patients with SPL.

Ricardo Marcos-Pinto and Ângela Fernandes share last authorship.

karger@karger.com
www.karger.com/pjg

Correspondence to:
Ricardo Marcos-Pinto, ricardomarcospinto@sapo.pt
Ângela Fernandes, angelaf@ipatimup.pt

Fatores de risco das lesões da via serreada: perfil de N-glicosilação como um potencial biomarcador de progressão para malignidade

Palavras Chave
Cancro colorretal · Via serreada · Lesões serreadas · *N*-glicosilação · *N*-glicanos β1,6-GlcNAc ramificados · Biomarcador

Resumo
Introdução: A via serreada contribui para os cancros colorretais de intervalo, destacando a necessidade de novos biomarcadores para determinar o risco de progressão destas lesões. A expressão de β1,6-GlcNAc *N*-glicanos ramificados foi associada a um fenótipo invasivo e a evasão imune. Assim, este estudo tem como objetivo identificar potenciais fatores de risco de progressão das lesões serreadas para malignidade, analisando o perfil de N-glicosilação das células epiteliais/células imunitárias. ***Métodos:*** Foi realizado um estudo retrospetivo com dados de 53 colonoscopias (48 doentes). 63 lesões da via serreada foram caracterizadas segundo o perfil de *N*-glicosilação (histoquímica de lectinas/citometria de fluxo) e expressão de *MGAT5*. A análise estatística foi realizada para encontrar associações entre o perfil de *N*-glicosilação e as variáveis clínicas de cada doente. ***Resultados:*** O aumento da expressão de β1,6-GlcNAc *N*-glicanos ramificados nas células epiteliais encontra-se associado com a idade (p = 0.007 nas SPL), tabagismo (p = 0.038 nas SL), aumento do BMI (p = 0.036 nas SSL), e pólipos com dimensões ≥10 mm (p = 0.001 nas SL), enquanto que o aumento destas estruturas nas células imunitárias está associado com o número de CA síncronos (células $TCD4^+$: p = 0.016; células $TCD8^+$: p = 0.044 nas SL) e o género feminino (p = 0.026 nas SL). Além disso, uma diminuição da expressão de N-glicanos ricos em manose está associada ao tabagismo (p = 0.010 para SPL) e a presença de adenomas síncronos (p = 0.010 nas SPL). A expressão aumentada destas estruturas está associado com o género feminino (p = 0.016 nas SSL), género masculino (p = 0.044 nas SSL), localização no cólon esquerdo (p = 0.028), displasia (p = 0–028) e adenocarcinoma (p = 0.010). ***Discussão/Conclusão:*** Identificámos uma associação entre um perfil de glicosilação anormal e vários fatores de risco clínicos, propondo o perfil de *N*-glicosilação como um potencial biomarcador de progressão tumoral na via serreada. A análise anatomopatológica do perfil de *N*-glicosilação pode vir a ser usada para decidir intervalos de follow-up mais curtos em doentes com SPL.

Introduction

Colorectal cancer (CRC) is the third most frequent cancer and it is responsible for 10% of cancer mortality worldwide [1, 2]. This cancer type occurs mostly from conventional adenoma (CA)-carcinoma pathway, while serrated pathway is responsible for about 25% of the cases [3]. Serrated lesions (SLs) are known as the precursor lesion in this carcinogenic pathway. SL can be divided into hyperplastic polyps (HPs), sessile serrated lesions (SSLs), sessile serrated lesions with dysplasia (SSLs-D), traditional serrated adenomas, and unclassified serrated adenomas, accordingly to the World Health Organization [4].

The last two decades were marked by advances in the study of the serrated pathway in order to understand the neoplastic mechanisms underlying this disease to prevent the progression to cancer [5]. This pathway is characterized by epigenetic alterations with mismatch repair genes deficiency and by the presence of a CpG island hypermethylation phenotype, with microsatellite instability in the vast majority of the cases [1, 6, 7]. Consequently, the serrated pathway presents a high lymphocytic immune infiltrate and upregulation of immune checkpoints associated with tumor immune evasion [8]. However, there is a gap of knowledge in understanding this pathway, particularly the progression to malignancy and the risk factors involved.

Some association studies defined smoking, alcohol consumption, overweight, red meat consumption, hypertension, and hypertriglyceridemia as risk factors for the SL development. Other researchers identified aging, absence of regular consumption of non-steroidal anti-inflammatory drugs, polyp dimensions ≥10 mm, dysplasia, female gender, and synchronous CA as risk factors for progression to malignancy in the serrated pathway [7, 9–15]. However, these risk factors are not as well established as in the adenoma-carcinoma pathway. Additionally, this CRC subtype is one of the responsibilities for the occurrence of interval cancers. This is presumed to be secondary to the difficult endoscopic identification of these lesions due to their sessile morphology, mucus coverage, and proximal colon location and rapid cancer progression after the development of dysplasia [7, 9, 10].

Thus, the protective effect of CRC screening is expected to decrease in these patients [7, 10]. This represents a challenge to physicians managing these cases due to the lack of biomarkers that could impact therapeutic decisions. Taking this into account, it seems crucial to identify a new biomarker capable of improving risk stratification and further clinical decision.

N-glycosylation has been associated with the malignant transformation process, and it is considered to be a cancer hallmark [11]. This process is a post-translational modification characterized by enzymatic reactions that allow the binding of carbohydrates (glycans) to proteins, lipids, or other saccharides [11]. These glycan structures are found on all cell surfaces, constituting the glycocalyx [12]. The differential glycans profiles are associated with immunologic and epithelial biologic functions [13]. In fact, our group described that the expression of β1,6-GlcNAc branched *N*-glycans in the conventional colorectal carcinogenesis cascade is considered an important immune checkpoint, demonstrating that these complex *N*-glycans overexpression in CRC cells was associated with immune escape [14]. Additionally, Demetriou et al. [15] demonstrated that T-cell activity is particularly regulated by β1,6-GlcNAc branched *N*-glycans on the T-cell receptor that modulates the threshold of T-cell activation and signaling. In line with this and in the context of chronic inflammatory processes such as inflammatory bowel disease, our group showed that these complex *N*-glycans are capable of regulating T-cell-mediated immune response associated with disease severity [16]. Particularly, we demonstrated that a β1,6-GlcNAc branched *N*-glycans deficiency, due to a *MGAT5* decreased expression, confers an hyperimmune response by decreasing T-cell activation threshold, increasing proinflammatory cytokines production, and increasing T-cell signaling [16]. This highlights the crucial role of the *N*-glycans pattern on cancer development/progression and immune response regulation. Therefore, in this study, we aimed to identify risk factors for progression to malignancy of serrated pathway lesions (SPLs) based on the *N*-glycosylation profile of both cancer cells and infiltrating immune cells.

Methods

Cohort Characterization

This is retrospective cohort study of patients with lesions of the serrated pathway, followed between September 2014 and 2021. Data were collected from 53 colonoscopies, corresponding to 48 patients.

The *N*-glycosylation profile was previously obtained from FFPE (formalin-fixed paraffin-embedded) biopsies and fresh biopsies of SPL. The *MGAT5* gene (gene that encodes the enzyme *N*-acetylglucosaminyltransferase-V [GnT-V]), responsible for the expression of β1,6-GlcNAc branched *N*-glycans, was evaluated by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) in FFPE biopsies. Also, in FFPE biopsies, a lectin histochemistry was performed to evaluate the expression of β1,6-GlcNAc branched *N*-glycans (complex glycans) in epithelial and stromal cells, obtained by staining with *Phaseolus vulgaris leucoagglutinin* (L-PHA), as well as the presence of high-mannose *N*-glycans (simple glycans), identified by labeling *Glanthus nivalis agglutinin*. The lectin histochemistry evaluation was performed by two independent observers, who gave a score from 0 to 3 according to the degree of staining (0: ≤25%; 1: 26% to 50%, 2: 51% to 75%, and 3: >75%). Flow cytometry of epithelial cells ($CD45^-$ cells), $CD4^+$ T cells, $CD8^+$ T cells, and $FoxP3^+CD25^+$ T cells (regulatory T cell – Treg) was performed on fresh biopsies, and L-PHA and *Glanthus nivalis agglutinin* expression was obtained, corresponding to β1,6-GlcNAc branched *N*-glycans and high-mannose *N*-glycans expression, respectively, in each of these cell populations.

Statistical Analysis

Descriptive statistics were performed based on the analysis of the mean and standard deviation of the continuous variables under study; percentages were used for the categorical variables. The relationship between the clinical variables and the *N*-glycosylation profile of the epithelial cells and colonic T cells obtained by RT-qPCR, lectin histochemistry, and flow cytometry was performed using Pearson's correlation, *t* test for independent samples, and nonparametric Mann-Whitney U. This relationship was performed for 3 groups: SPL (which includes SL and serrated pathway adenocarcinoma), SL (which includes HP, SSL, and SSL-D), and SSL (with or without dysplasia). The association between continuous variables and non-binary discrete variables was performed using the one-way ANOVA test, using Tukey's post hoc test. A significance level of 0.05 was considered and the statistical analysis of the variables was performed using the SPSS version 26 program.

Results

Of the 53 colonoscopies performed, 63 samples of SPL were obtained (34 FFPE biopsies and 29 fresh biopsies). This cohort includes 44 (69.8%) SSL without dysplasia, 9 (14.3%) SSL-D, 7 (11.1%) HP, 1 (1.6%) adenocarcinoma of the serrated pathway, and 2 (3.2%) SSL-D with concomitant adenocarcinoma (Table 1). The description of the sample regarding the remaining sociodemographic characteristics, risk factors, and anatomopathological characteristics is depicted in detail in Table 1. The descriptive statistic of *N*-glycosylation profile is depicted in detail in Table 2.

Altered Premalignant Epithelial N*-Glycosylation Profile Is Correlated with Age, Smoking, Increased BMI, Polyp's Dimensions, and Lesion Location in the Serrated Pathway*

The *N*-glycosylation profile of epithelial cells was correlated with potential risk factors for disease progression in the serrated pathway. Our results indicated a

Table 1. Cohort characterization

Variable	*N* (%)	Mean	Standard deviation
Gender	48		
Male	24 (50.0)		
Female	24 (50.0)		
Age, years		64.2	11.4
Smoking	48		
No	25 (52.1)		
Yes	23 (47.9)		
Missing	0 (0)		
BMI, kg/m^2	42	27.3	4.9
Lesion number		3.6	4.5
SPL number		3.1	4.4
Synchronous CA number		0.6	1.2
Synchronous CA			
No	47 (74.6)		
Yes	16 (25.4)		
Classification	63		
SSL without dysplasia	44 (69.8)		
SSL-D	9 (14.3)		
HP	7 (11.1)		
Adenocarcinoma	1 (1.6)		
Adenocarcinoma + SSL-D	2 (3.2)		
Location	63		
Right colon	41 (65.1)		
Transverse colon	15 (23.8)		
Left colon	7 (11.1)		
Missing	0 (0)		
Dimension, mm	63	22.8	11.4
<10	4 (6.3)		
≥10	58 (92.1)		
Missing	1 (1.6)		

BMI, body mass index; CA, conventional adenoma; HP, hyperplastic polyp; SPL, serrated pathway lesion; SSL, sessile serrated lesion; SSL-D, sessile serrated lesion with dysplasia.

significant correlation between increased age and the β1,6-GlcNAc branched *N*-glycans expression (L-PHA expression) on epithelial cells in SPL ($p = 0.007$; $r = 0.501$) (Table 3). Additionally, tobacco consumption was also associated with an increased *MGAT5* gene expression. In fact, smokers presented an evident higher β1,6-GlcNAc branched *N*-glycans expression in SL comparing to non-smokers (Δct value: $2.50 \times 10^{-4} \pm 2.62 \times 10^{-4}$ vs. $9.49 \times 10^{-5} \pm 9.82 \times 10^{-5}$; $p = 0.038$) (Table 4). Furthermore, a statistically significant correlation was also observed between body mass index (BMI) and β1,6-GlcNAc branched *N*-glycans expression in SPL ($p = 0.045$; $r = 0.432$) and in SSL ($p = 0.036$; $r = 0.496$), regarding *MGAT5* gene expression (Table 4). In addition, our data indicate a higher β1,6-GlcNAc branched *N*-glycans expression in SL with dimensions ≥10 mm, comparing to SL with dimensions <10 mm, regarding *MGAT5* gene expression (Δct value: $1.70 \times 10^{-4} \pm 1.68 \times 10^{-4}$ vs. $4.40 \times 10^{-5} \pm 3.96 \times 10^{-6}$; $p = 0.001$) (Table 4). Concerning the SPLs location, there was an increase in the high-mannose *N*-glycans expression in the epithelial part of the left colon, compared to the right and transverse colon (2.50 ± 0.71 vs. 0.80 ± 0.84 vs. 0.40 ± 0.22; $p = 0.009$) (Table 4).

Altered Immune N-*Glycosylation Profile Is Correlated with Increased Synchronous CA, Sex, Smoking, Location of the Lesion, and BMI in the Serrated Pathway*

The *N*-glycosylation profile of immune cells was correlated with potential risk factors for disease progression in the serrated pathway. Our results demonstrated a positive correlation between the synchronous CA number in patients with SPL, SL, and SSL and β1,6-GlcNAc branched *N*-glycans expression in CD4$^+$ T cells ($p = 0.022$, $r = 0.475$; $p = 0.025$, $r = 0.477$; $p = 0.044$, $r = 0.493$) and CD8$^+$ T cells ($p = 0.014$, $r =$

Table 2. Statistical description of the glycosylation profile of SPLs

	Mean±standard deviation	Median	Minimum	Maximum
MGAT5 gene RT-qPCR (Δct value)	$2.6 \times 10^{-4} \pm 4.4 \times 10^{-4}$	9.30017×10^{-5}	1.14743×10^{-5}	2.37524×10^{-3}
Histochemistry				
Epithelial L-PHA	1.2±0.9	1.25	0	2
Stromal L-PHA	0.9±0.6	1	0	2
Epithelial GNA	0.9±0.9	0.5	0	3
Stromal GNA	1.6±0.9	1	0.5	3
FC				
Epithelial L-PHA	233.1±156.9	185	20.6	713
Epithelial GNA	148.4±129.9	109	25.7	551
T $CD4^+$ L-PHA	984.4±805.0	838.5	134	3,497
T $CD4^+$ GNA	132.1±146.6	67.5	0	547
T $CD8^+$ L-PHA	1,376.8±1,076.3	1,049	413	4,416
T $CD8^+$ GNA	558.2±965.8	73.2	0	2,843
FoxP3 T $CD25^+$ L-PHA	1,906.7±1,960.7	1,742.5	223	8,775
FoxP3 T $CD25^+$ GNA	1,172.3±2,138.0	559	50.1	9,862

FC, flow cytometry; GNA, *Glanthus nivalis agglutinin*; L-PHA, *Phaseolus vulgaris leucoagglutinin*; RT-PCR, reverse transcriptase-quantitative polymerase chain reaction.

0.602; $p = 0.008$, $r = 0.655$; $p = 0.005$, $r = 0.727$) (Table 3). Furthermore, synchronous CA in patients with SPL was associated with a lower high-mannose *N*-glycans expression in the stromal cells of SPL (0.75 ± 0.25 vs. 1.83 ± 0.94; $p = 0.010$) and specifically in $CD4^+$ T cells in patients with SSL (44.70 ± 13.82 vs. 134.44 ± 157.42; $p = 0.025$) (Tables 3, 4). Furthermore, smokers showed, on average, a lower high-mannose *N*-glycans expression in the SPL stromal cells, when compared with non-smokers (1.19 ± 0.73 vs. 2.67 ± 0.58; $p = 0.010$) (Table 4). Regarding the BMI, we observed an inverse relationship between this factor and the expression of high-mannose *N*-glycans in Tregs in SPL and SL ($p = 0.034$, $r = -0.487$; $p = 0.042$, $r = -0.484$) (Table 3). Additionally, females presented, on average, a higher high-mannose *N*-glycans expression in $CD4^+$ T cells in SPL (178.86 ± 164.04 vs. 67.98 ± 50.69; $p = 0.020$), in SSL (299.87 ± 179.86 vs. 44.08 ± 29.48; $p = 0.017$), and in SL (178.86 ± 164.04 vs. 52.65 ± 38.99; $p = 0.016$) comparing to males (Table 3). Moreover, females showed, on average, a higher β1,6-GlcNAc branched *N*-glycans expression on Tregs in SPL (2,556.36 ± 2,269.88 vs. 1,036.99 ± 990.22; $p = 0.040$), in SL (2,736.70 ± 2,308.10 vs. 1,036.99 ± 990.21; $p = 0.026$), and in SSL (3,005.86 ± 2,759.88 vs. 916.18 ± 813.00; $p = 0.029$), comparing with males (Table 3). On the other hand, males presented, on average, a higher high-mannose *N*-glycans expression in $CD8^+$ T cells in SPL (778.79 ± 1,084.28 vs. 67.98 ± 50.69; $p = 0.044$), in SSL (884.16 ± 1,112.12 vs. 65.55 ± 57.13; $p = 0.043$), and in SL (778.86 ± 1,084.28 vs. 67.78 ± 50.69; $p = 0.044$) (Table 3). Furthermore, left colon lesions presented a higher high-mannose *N*-glycans expression in stromal cells compared to the transverse colon (3.00 ± 0.00 vs. 1.20 ± 0.57; $p = 0.039$) (Table 4). SPL with dysplasia showed a higher high-mannose *N*-glycans expression in stromal cells (2.17 ± 0.98 vs. 0.96 ± 0.33; $p = 0.028$), comparing with non-dysplastic SPL (Table 4). Also, in serrated pathway adenocarcinomas and SSL-D with concomitant adenocarcinoma a higher high-mannose *N*-glycans expression on stroma was observed on average (2.67 ± 0.80 vs. 1.19 ± 0.73; $p = 0.010$) (Table 4).

Discussion

SPL follow-up still raises serious concerns due to rapid progression from dysplasia to cancer. Moreover, there are few robust studies of clinical progression risk factors on serrated pathway. The changes in *N*-glycosylation has been considered as a CRC progression hallmark in epithelial cells [11]. Thus, the main goal of our study was to define potential risk factors for progression to malignancy by analyzing the *N*-glycosylation profile of the serrated pathway.

We found an increased β1,6-GlcNAc branched *N*-glycans expression in the SPL epithelial component correlated with increasing age and BMI. In SL, β1,6-GlcNAc branched *N*-glycans expression in the

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 3. N-glycosylation profile results obtained by FC

Variable	Epithelial L-PHA			Epithelial GNA			T CD4+ L-PHA			T CD4+ GNA			T CD8+ L-PHA			T CD8+ GNA			Treg L-PHA			Treg GNA		
	N	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value
Gender																								
SPL			0.308			0.443			0.396			**0.014**			0.309			**0.044**			**0.040**			0.980
Male	13	204.62± 90.35		13	139.35± 86.77		13	830.54± 392.51		**14**	**52.65± 38.99**		10	1,182.60± 432.40		**12**	**778.79± 1,084.28**		**13**	**1,036.99± 990.21**		13	1,028.22± 2,679.55	
Female	15	259.87± 171.95		14	176.89± 152.10		13	1,073.85± 935.95		**14**	**178.86± 164.04**		9	1,608.78± 1,204.08		**10**	**67.98± 50.69**		**11**	**2,556.36± 2,269.88**		12	1,049.29± 886.34	
SL			0.587			0.386			0.404			**0.016**			0.195			**0.044**			**0.026**			0.907
Male	13	204.62± 90.35		13	139.35± 86.77		13	830.54± 392.51		**14**	**52.65± 38.99**		10	1,182.60± 432.40		**12**	**778.79± 1,084.28**		**13**	**1,036.99± 990.21**		13	1,028.22± 2,679.55	
Female	14	227.50± 122.14		13	183.19± 156.41		12	1,079.83± 977.31		**13**	**185.30± 168.89**		8	1,739.25± 1,217.31		**10**	**67.98± 50.69**		**10**	**2,736.70± 2,308.10**		11	1,128.50± 883.94	
SSL			0.822			0.369			0.309			**0.017**			0.119			**0.043**			**0.029**			0.958
Male	11	223.76± 77.05		11	148.80± 87.38		11	820.09± 322.43		**12**	**44.083± 29.48**		9	1,161.11± 452.93		**11**	**844.16± 1,112.12**		**11**	**916.18± 813.00**		11	1,138.10± 2,916.71	
Female	11	233.64± 121.67		11	200.48± 164.97		9	1,187.78± 114.37		**11**	**200.37± 179.86**		7	1,892.14± 1,229.08		**8**	**65.55± 57.13**		**7**	**3,005.86± 2,759.88**		9	1,083.39± 918.62	
Age, years																								
SPL	**28**	**0.501***	**0.007**	27	−0.107*	0.596	26	0.206*	0.314	28	−0.183*	0.352	19	−0.101*	0.682	22	0.136*	0.547	24	0.167*	0.436	25	−0.067*	0.751
SL	**27**	**0.422***	**0.028**	26	−0.080*	0.697	25	0.212*	0.308	27	−0.182*	0.363	18	−0.028*	0.913	22	0.136*	0.547	23	0.218*	0.317	24	−0.041*	0.848
SSL	22	0.224*	0.316	22	−0.227*	0.309	20	0.224*	0.343	23	−0.166*	0.449	16	−0.058*	0.830	19	0.104*	0.672	18	0.242*	0.334	20	0.002*	0.993
Smoking																								
SPL			0.195			0.773			0.281			0.715			0.763			0.629			0.357			0.179
No	14	269.07± 143.48		14	151.97± 105.63		13	1,106.08± 922.12		14	106.28± 126.00		11	1,439.00± 1,050.68		11	263.58± 656.57		11	1,349.36± 1,043.60		12	475.91± 721.11	
Yes	14	199.26± 133.45		13	166.19± 145.55		13	798.31± 400.64		14	125.23± 144.70		8	1,309.50± 657.32		11	547.81± 1,058.95		13	2,057.31± 2,294.65		13	1,557.49± 2,610.63	
SL			0.397			0.847			0.287			0.737			0.622			0.629			0.420			0.210
No	13	234.92± 67.93		13	156.35± 108.61		12	1,114.75± 962.57		13	107.14± 131.10		10	1,526.40± 1,064.53		11	363.58± 656.57		10	1,049.00± 1,080.11		11	502.99± 749.88	
Yes	14	199.36± 133.45		13	166.19± 145.55		13	798.31± 400.64		14	125.23± 144.70		8	1,309.50± 657.32		11	547.81± 1,058.95		13	2,057.31± 2,294.65		13	1,557.49± 2,610.63	
SSL			0.783			0.633			0.251			0.783			0.809			0.549			0.410			0.213
No	12	223.17± 55.45		12	161.98± 111.44		11	1,171.46± 988.31		12	110.50± 136.34		10	1,526.40± 1,064.53		10	391.72± 685.06		9	1,317.67± 1,103.91		10	489.49± 789.03	
Yes	10	235.34± 138.77		10	189.83± 157.19		9	758.33± 358.98		11	127.92± 163.41		6	1,405.17± 714.84		9	654.78± 1,153.65		9	2,140.00± 2,697.21		10	1,737.47± 2,952.34	
BMI, kg/m^2																								
SPL	22	−0.061*	0.787	21	−0.410*	0.065	21	−0.012*	0.958	22	−0.397*	0.067	18	0.142*	0.574	16	−0.242*	0.366	19	−0.230*	0.344	**19**	**−0.487***	**0.034**
SL	21	−0.155*	0.502	20	−0.405*	0.076	20	−0.012*	0.959	21	−0.398*	0.074	17	0.161*	0.537	16	−0.242*	0.366	18	−0.224*	0.371	**18**	**−0.484***	**0.042**
SSL	18	−0.275*	0.270	18	−0.390*	0.110	17	−0.021*	0.937	19	−0.392*	0.097	15	0.131*	0.643	15	−0.216*	0.440	15	−0.292*	0.291	16	−0.489*	0.055
Dimension, mm																								
SPL			–			–			–			–			–			–			–			–
<10	0	–		0	–		0	–		0	–		0	–		0	–		0	–		0	–	
≥10	27	216.48± 106.59		26	161.27± 125.92		25	950.20± 728.67		27	116.52± 135.96		18	1,430.00± 888.91		22	455.70± 864.96		23	1,775.44± 1,859.38		24	1,074.18± 2,021.98	
SL			–			–			–			–			–			–			–			–
<10	0	–		0	–		0	–		0	–		0	–		0	–		0	–		0	–	
≥10	27	216.48± 106.59		26	161.27± 125.92		25	950.20± 728.67		27	116.52± 135.96		18	1,430.00± 888.91		22	455.70± 864.96		23	1,775.44± 1,859.38		24	1,074.18± 2,021.98	

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 3 (continued)

Variable	Epithelial L-PHA			Epithelial GNA			T CD4+ L-PHA			T CD4+ GNA			T CD8+ L-PHA			T CD8+ GNA			Treg L-PHA			Treg GNA		
	N	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value
SSL			–			–			–			–			–			–			–			-
<10	0	–		0	–		0	–		0	–		0	–		0	–		0	–		0	–	
≥10	22	228.70± 99.51		22	174.64± 131.51		20	985.55± 782.82		23	118.83± 146.67		16	1,480.94± 924.09		19	516.33± 918.90		18	1,728.83± 2,043.52		20	1,113.48± 2,198.53	
Dysplasia																								
SPL			–			–			–			0.312			–			0.548			–			–
No	26	213.19± 107.30		25	161.48± 128.51		24	948.96± 744.32		25	124.14± 138.46		17	1,437.35± 915.70		20	491.86± 900.91		22	1,827.23± 1,886.08		23	1,119.37± 2,054.99	
Yes	1	302.00		1	156.00		1	980.00		2	21.20± 29.98		1	1,305.00		2	94.05± 49.43		1	636.00		1	34.70	
SL			–			–			–			0.312			–			0.548			–			–
No	26	213.19± 107.30		25	161.48± 128.51		24	948.96± 744.32		25	124.14± 138.46		17	1,437.35± 915.70		20	491.86± 900.91		22	1,827.23± 1,886.08		23	1,119.37± 2,054.99	
Yes	1	302.00		1	156.00		1	980.00		2	21.20± 29.98		1	1,305.00		2	94.05± 49.43		1	636.00		1	34.70	
SSL			–			–			–			0.336			–			0.508			–			–
No	21	225.21± 100.58		21	175.53± 134.69		19	985.84± 804.27		21	128.13± 150.24		15	1,492.67± 955.29		17	566.01± 961.70		17	1,793.12± 2,087.57		19	1,170.26± 2,243.66	
Yes	1	302.00		1	156.00		1	980.00		2	21.20± 29.98		1	1,305.00		2	94.05± 49.43		1	636.00		1	34.70	
Synchronous CA																								
SPL			0.699			0.171			0.265			0.096			0.433			0.051			0.398			0.219
No	21	228.11± 111.51		20	178.38± 137.86		19	855.74± 514.56		21	131.39± 150.07		14	1,227.86± 536.31		18	540.11± 939.40		17	1,940.65± 2,026.58		18	1,347.82± 2,277.49	
Yes	7	252.51± 216.53		7	102.01± 40.83		7	1,214.00± 1,104.78		7	68.86± 41.06		5	1,832.00± 1,508.10		4	75.85± 18.89		7	1,228.14± 1,213.32		7	242.51± 279.52	
SL			0.298			0.212			0.469			0.297			0.325			0.051			0.486			0.260
No	21	228.11± 111.51		20	178.38± 137.86		19	855.74± 514.56		21	131.39± 150.07		14	1,227.86± 536.31		18	540.11± 939.40		17	1,940.65± 2,026.58		18	1,347.82± 2,277.49	
Yes	6	175.77± 82.36		6	104.22± 44.57		6	1,249.33± 1,205.89		6	64.48± 43.15		4	2,137.500± 1,540.48		4	75.85± 18.89		6	1,307.33± 1,309.16		6	253.27± 304.61	
SSL			0.753			0.309			0.496			**0.025**			0.390			0.053			0.511			0.350
No	18	231.97± 109.25		18	188.42± 142.03		16	874.44± 552.45		**19**	**134.44± 157.42**		13	1,270.85± 532.51		16	597.91± 984.00		14	1,904.64± 2,237.02		16	1,349.86± 2,411.30	
Yes	4	214.00± 36.47		4	112.65± 22.18		4	1,430.00± 1,423.34		**4**	**44.70± 13.82**		3	2,391.33± 1,781.31		3	81.23± 19.02		4	1,113.50± 1,153.06		4	167.98± 225.08	
Synchronous CA number																								
SPL	25	−0.031*	0.882	24	−0.256*	0.228	**23**	**0.475***	**0.022**	25	−0.274*	0.185	**16**	**0.602***	**0.014**	19	−0.116*	0.637	21	−0.134*	0.562	22	−0.282*	0.204
SL	24	−0.162*	0.449	23	−0.245*	0.260	**22**	**0.477***	**0.025**	24	−0.271*	0.201	**15**	**0.655***	**0.008**	19	−0.116*	0.637	20	−0.121*	0.612	21	−0.273*	0.231
SSL	19	−0.120*	0.625	19	−0.264*	0.274	**17**	**0.493***	**0.044**	20	−0.272*	0.246	**13**	**0.727***	**0.005**	16	−0.118*	0.664	15	−0.162*	0.564	17	−0.247*	0.339
Location																								
SPL			0.538			0.739			0.068			0.382			0.022			0.390			0.726			0.928
Right colon	19	254.63± 165.58		18	155.18± 124.04		18	749.67± 379.37		19	91.29± 120.16		14	1,068.14± 430.38		15	632.67± 1,007.77		18	1,574.50± 2,021.34		18	1,072.38± 2,288.92	
Transverse colon	7	197.14± 46.55		7	183.21± 145.79		7	1,476.29± 1,128.23		7	164.36± 163.98		4	2,238.75± 1,459.49		5	96.64± 44.70		5	2,078.00± 1,150.33		5	1,131.48± 1,086.42	
Left colon	2	170.00± 16.97		2	106.10± 39.46		1	929.00		2	178.05± 155.49		1	2,396.00		2	26.00± 36.77		1	2,857.00		2	499.05± 609.46	

Table 3 (continued)

Variable		Epithelial L-PHA			Epithelial GNA			T CD4+ L-PHA			T CD4+ GNA			T CD8+ L-PHA			T CD8+ GNA			Treg L-PHA			Treg GNA		
	N	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value	*n*	mean	*p* value
SL			0.666			0.755			0.071			0.401			0.033			0.390			0.764			0.922
Right colon	18	229.17± 126.43		17	158.72± 126.92		17	734.82± 385.62		18	91.08± 123.64		13	1,106.85± 421.83		15	632.67± 1,007.77		17	1,622.82± 2,071.80		17	1,124.99± 2,348.12	
Transverse colon	7	197.14± 46.55		7	183.21± 145.79		7	1,476.29± 1,128.23		7	164.36± 163.98		4	2,238.75± 1,459.49		5	96.64± 44.70		5	2,078.00± 1,150.33		5	1,131.48± 1,086.42	
Left colon	2	170.00± 16.97		2	106.10± 39.46		1	929.00		2	178.05± 155.49		1	2,396.00		2	26.00± 36.77		1	2,857.00		2	499.05± 609.46	
SSL			0.537			0.707			0.101			0.479			0.058			0.387			0.825			0.892
Right colon	14	245.53± 119.87		14	173.91± 133.54		13	730.00± 350.15		15	90.97± 134.94		11	1,122.18± 432.27		13	719.09± 1,060.26		13	1,571.92± 2,312.86		14	1,259.94± 2,579.78	
Transverse colon	6	209.00± 39.14		6	199.18± 152.85		6	1,548.67± 1,217.98		6	168.75± 179.17		4	2,238.75± 1,459.49		4	102.50± 49.35		4	1,956.75± 1,290.87		4	908.10± 1,114.04	
Left colon	2	170.00± 16.97		2	106.10± 39.46		1	929.00		2	178.05± 155.29		1	2,396.00		2	26.00± 36.77		1	2,857.00		2	499.05± 609.46	
Adenocarcinoma SPL																									
No	27	216.48± 106.59		26	161.27± 125.92		25	959.20		27	116.52± 135.96		18	1,430.00± 888.91		22	455.70± 864.96		23	1,775.44± 1,859.38		24	1,074.18± 2,021.98	
Yes	1	713.00		1	95.10		1	1,002.00		1	95.10		1	565.00		0	–		1	753.00		1	178.00	

BMI, body mass index; CA, conventional adenoma; GNA, *Glanthus nivalis agglutinin*; L-PHA, *Phaseolus vulgaris leucoagglutinin*; SL, serrated lesion; SPL, serrated pathway lesion; SSL, sessile serrated lesion; *r*, correlation coefficient; Treg, regulatory T cell; FC, flow cytometry. *Correlation coefficient.

epithelial component is also correlated with smoking and polyp dimensions ≥10 mm. Previously, our group showed that these types of glycans present an aberrant expression in CRC and are direct immune modulators in the tumor microenvironment, allowing immune evasion [14]. Thus, these clinical variables may intervene as risk factors for the progression to malignancy in the serrated pathway by enabling identification of immunological escape in these lesions. In fact, age is a known risk factor for the CRC development and progression, with immunosenescence potentially playing an important role in the immune escape suggested in our results [1, 17]. Smoking is a studied and validated risk factor for SL development and progression to CRC [17, 18]. This evidence is in line with our results, emphasizing smoking cessation as a method of preventing progression in the serrated pathway. Additionally, BMI has been described in the literature as a possible risk factor for CRC progression [19]. However, it is not fully understood whether it impacts the adenoma-carcinoma pathway or the serrated pathway [19]. Our findings suggest that BMI may contribute to progression to malignancy in the serrated pathway by upregulating β1,6-GlcNAc branched *N*-glycans in epithelial cells. Thereby, weight loss should be encouraged in individuals with SPL in an attempt to prevent progression to malignancy. Regarding SL dimensions, our results suggest that polyps with dimensions ≥10 mm have greater risk of progression to malignancy, by overexpression of β1,6-GlcNAc branched *N*-glycans in epithelial cells. This result is in line with the European guideline for post-polypectomy colonoscopy follow-up, which defined a cut-off of 10 mm to perform a shorter interval follow-up [20].

Regarding the immune compartment, it was observed a higher β1,6-GlcNAc branched *N*-glycans expression with the increasing number of synchronous CA in patients with SPL. Previously, our group had shown that an increasing β1,6-GlcNAc branched *N*-glycans expression in T cells, by GlcNAc supplementation, controls T-cell immune response in inflammatory bowel disease, by increasing its threshold of activation [16]. Thus, T-cell β1,6-GlcNAc branched *N*-glycosylation seems to create an immunosuppressive environment disabling T-cell activation and function in SPL, promoting their growth. This immunosuppression promotes the development of more synchronous CA. Therefore, a higher synchronous CA number seems to be a risk factor for progression to malignancy in the serrated pathway. Synchronous CA number has not been described in the literature as a risk factor for development or progression to malignancy in the serrated pathway yet. Thereby, the anatomopathological identification of the *N*-glycosylation pattern in the presence of a high synchronous CA number can select patients that would

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 4. N-glycosylation profile results obtained by MGAT5 gene RT-qPCR and histochemistry

Variable	*MGAT5* gene RT-qPCR			Epithelial L-PHA			Epithelial GNA			Stromal L-PHA			Stromal GNA		
	N	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value
Gender															
SPL			0.124			0.338			0.387			0.628			0.943
Male	16	$3.83 \times 10^{-4} \pm 5.97 \times 10^{-4}$		6	0.92±0.92		6	1.17±1.13		6	0.83±0.52		6	1.54±1.14	
Female	17	$1.35 \times 10^{-4} \pm 1.31 \times 10^{-4}$		6	1.42±0.80		6	0.67±0.75		6	1.00±0.63		6	1.58±0.80	
SL			0.240			0.102			0.677			0.601			0.172
Male	14	$1.37 \times 10^{-4} \pm 1.35 \times 10^{-4}$		4	0.88±0.85		4	0.50±0.41		4	0.75±0.65		4	0.81±0.24	
Female	16	$2.39 \times 10^{-4} \pm 2.88 \times 10^{-4}$		5	1.70±0.45		5	0.70±0.84		5	1.00±0.71		5	1.50±0.87	
SSL			0.273			0.143			0.725			0.553			0.205
Male	14	$1.43 \times 10^{-4} \pm 1.37 \times 10^{-4}$		3	0.83±1.04		3	0.50±0.50		3	0.67±0.76		3	0.75±0.25	
Female	15	$2.39 \times 10^{-4} \pm 2.88 \times 10^{-4}$		5	1.70±0.45		5	0.70±0.84		5	1.00±0.71		5	1.50±0.87	
Age, years															
SPL	33	0.139*	0.440	12	−0.361*	0.263	12	−0.261*	0.650	12	0.146*	0.412	12	0.040*	0.901
SL	30	0.017*	0.930	9	−0.454*	0.219	9	−0.329*	0.310	9	−0.382*	0.387	9	−0.479*	0.192
SSL	29	0.016*	0.933	8	−0.451*	0.262	8	−0.335*	0.354	8	−0.379*	0.418	8	−0.476*	0.233
Smoking															
SPL			0.878			0.265			0.106			0.780			**0.010**
No	15	$2.68 \times 10^{-4} \pm 5.95 \times 10^{-4}$		3	0.67±1.15		3	1.84±1.26		3	1.00±0.00		**3**	**2.67±0.58**	
Yes	18	$2.45 \times 10^{-4} \pm 2.62 \times 10^{-4}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		**9**	**1.19±0.73**	
SL			**0.038**			–			–	–		–			–
No	**12**	$\mathbf{9.49 \times 10^{-5} \pm 9.82 \times 10^{-5}}$		0	–		0	–		0	–		0	–	
Yes	**18**	$\mathbf{2.50 \times 10^{-4} \pm 2.62 \times 10^{-4}}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		9	1.19±0.73	
SSL			0.053			–			–			–			–
No	11	$9.99 \times 10^{-5} \pm 1.01 \times 10^{-4}$		0	–		0	–		0	–		0	–	
Yes	18	$2.45 \times 10^{-4} \pm 2.62 \times 10^{-4}$		8	1.38±0.79		8	0.63±0.69		8	0.88±0.69		8	1.22±0.77	
BMI, kg/m^2															
SPL	**22**	**0.432***	**0.045**	8	0.458*	0.254	8	−0.115*	0.516	8	0.271*	0.786	8	0.371*	0.445
SL	19	0.428*	0.068	5	0.566*	0.320	5	−0.115*	0.178	5	−0.711*	0.853	5	−0.032*	0.959
SSL	**18**	**0.496***	**0.036**	4	0.698*	0.302	4	−0.154*	0.236	4	−0.764*	0.846	4	−0.005*	0.995
Dimension, mm															
SPL			0.427			–			–			–			–
<10	3	$4.40 \times 10^{-5} \pm 3.96 \times 10^{-6}$		0			0	–		0	–		0	–	
≥10	29	$2.52 \times 10^{-4} \pm 4.40 \times 10^{-4}$		12	1.17±0.86		12	0.92±0.95		12	0.92±0.56		12	1.56±0.94	
SL			**0.001**			–			–			–			–
<10	**3**	$\mathbf{4.40 \times 10^{-5} \pm 3.96 \times 10^{-6}}$		0	–		0	–		0	–		0	–	
≥10	**26**	$\mathbf{1.70 \times 10^{-4} \pm 1.68 \times 10^{-4}}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		9	1.19±0.73	

Table 4 (continued)

Variable	*MGAT5* gene RT-qPCR			Epithelial L-PHA			Epithelial GNA			Stromal L-PHA			Stromal GNA		
	N	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value
SSL			0.316			–			–			–			–
<10	2	$4.61 \times 10^{-5} \pm 2.26 \times 10^{-6}$		0	–		0	–		0	–		0	–	
≥10	26	$1.70 \times 10^{-4} \pm 1.68 \times 10^{-4}$		8	1.38±0.79		8	0.63±0.69		8	0.88±0.69		8	1.22±0.77	
Dysplasia															
SPL			0.084			0.755			0.241			1			**0.028**
No	24	$1.75 \times 10^{-4} \pm 2.38 \times 10^{-4}$		6	1.08±0.80		6	0.58±0.38		6	0.92±0.80		**6**	**0.96±0.33**	
Yes	9	$4.70 \times 10^{-4} \pm 7.28 \times 10^{-4}$		6	1.25±0.99		6	1.25±1.26		6	0.92±0.20		**6**	**2.17±0.98**	
SL			0.637			0.170			0.913			0.870			0.800
No	24	$1.75 \times 10^{-4} \pm 2.38 \times 10^{-4}$		6	1.08±0.80		6	0.58±0.38		6	0.92±0.80		6	0.96±0.33	
Yes	6	$2.24 \times 10^{-4} \pm 1.51 \times 10^{-4}$		3	1.83±0.29		3	0.67±1.15		3	0.83±0.29		3	1.67±1.15	
SSL			0.683			0.229									
No	23	$1.81 \times 10^{-4} \pm 2.42 \times 10^{-4}$		5	1.10±0.89		5	0.60±0.42		5	0.90±0.89		5	0.95±0.37	
Yes	6	$2.24 \times 10^{-4} \pm 1.51 \times 10^{-4}$		3	1.83±0.29		3	0.68±1.15		3	0.83±0.29		3	1.67±1.15	
Synchronous CA															
SPL			0.646			0.265			0.406			0.780			**0.010**
No	23	$2.79 \times 10^{-4} \pm 5.15 \times 10^{-4}$		9	1.33±0.87		9	1.06±1.04		9	0.94±0.53		**9**	**1.83±0.94**	
Yes	10	$2.02 \times 10^{-4} \pm 1.60 \times 10^{-4}$		3	0.67±0.76		3	0.50±0.50		3	0.83±0.76		**3**	**0.75±0.25**	
SL			0.778			0.208			0.743			0.870			0.214
No	20	$1.76 \times 10^{-4} \pm 2.51 \times 10^{-4}$		6	1.67±0.52		6	0.67±0.75		6	0.92±0.66		6	1.42±0.80	
Yes	10	$2.01 \times 10^{-4} \pm 1.60 \times 10^{-4}$		3	0.67±0.76		3	0.50±0.50		3	0.83±0.76		3	0.75±0.25	
SSL			0.845			**0.035**			0.725			0.907			0.205
No	19	$1.84 \times 10^{-4} \pm 2.56 \times 10^{-4}$		**5**	**1.80±0.45**		5	0.70±0.84		5	0.90±0.74		5	1.50±0.87	
Yes	10	$2.01 \times 10^{-4} \pm 1.60 \times 10^{-4}$		**3**	**0.68±0.76**		3	0.50±0.50		3	0.83±0.76		3	0.75±0.25	
Synchronous CA number															
SPL	27	−0.074*	0.713	10	−0.308*	0.387	10	−0.551*	0.621	10	−0.179*	0.099	10	−0.501*	0.140
SL	25	0.037*	0.861	8	−0.607*	0.110	8	−0.569*	0.770	8	−0.124*	0.141	8	−0.481*	0.227
SSL	25	0.037*	0.861	7	−0.658*	0.108	7	−0.583*	0.742	7	−0.154*	0.170	7	**−0.522***	0.230
Location															
SPL	33		0.332	12		0.567	**12**		**0.009**	12		0.855	**12**		**0.039**
Right colon	20	$1.90 \times 10^{-4} \pm 2.29 \times 10^{-4}$		5	1.50±0.87		**5**	**0.80±0.84**		5	1.00±0.35		**5**	**1.35±0.93**	
Transverse colon	7	$2.35 \times 10^{-4} \pm 2.42 \times 10^{-4}$		5	0.90±0.74		**5**	**0.40±0.22**		5	0.80±0.84		**5**	**1.20±0.57**	
Left colon	6	$4.96 \times 10^{-4} \pm 9.31 \times 10^{-4}$		2	1.00±1.41		**2**	**2.50±0.71**		2	1.00±0.00		**2**	**3.00±0.00**	

GE Port J Gastroenterol 2024;31:338–350
DOI: 10.1159/000535920

Table 4 (continued)

Variable	*MGAT5* gene RT-qPCR			Epithelial L-PHA			Epithelial GNA			Stromal L-PHA			Stromal GNA		
	N	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value	*n*	mean/*r**	*p* value
SL			0.362			–			–			–			–
Right colon	20	$1.90 \times 10^{-4} \pm 2.29 \times 10^{-4}$		5	1.50±0.87		5	0.80±0.84		5	1.00±0.35		5	1.35±0.93	
Transverse colon	6	$2.55 \times 10^{-4} \pm 2.59 \times 10^{-4}$		4	1.13±0.63		4	0.38±0.25		4	0.75±0.96		4	1.00±0.41	
Left colon	4	$4.95 \times 10^{-5} \pm 4.61 \times 10^{-6}$		0	–		0	–		0	–		0	–	
SSL			0.362			–			–			–			–
Right colon	19	$1.98 \times 10^{-4} \pm 2.32 \times 10^{-4}$		5	1.50±0.87		5	0.80±0.84		5	1.00±0.35		5	1.35±0.93	
Transverse colon	6	$2.55 \times 10^{-4} \pm 2.59 \times 10^{-4}$		3	1.17±0.76		3	0.33±0.29		3	0.67±1.15		3	1.00±0.50	
Left colon	4	$4.50 \times 10^{-5} \pm 4.61 \times 10^{-5}$		0	–		0	–		0	–		0	–	
Adenocarcinoma															**0.010**
SPL			0.388			0.265			0.106			0.780			
No	30	$1.85 \times 10^{-4} \pm 2.22 \times 10^{-4}$		9	1.33±0.75		9	0.61±0.65		9	0.89±0.65		**9**	**1.19±0.73**	
Yes	3	$9.63 \times 10^{-4} \pm 1.23 \times 10^{-3}$		3	0.67±1.15		3	1.83±1.26		3	1.00±0.00		**3**	**2.67±0.58**	

BMI, body mass index; CA, conventional adenoma; GNA, *Glanthus nivalis agglutinin*; L-PHA, *Phaseolus vulgaris leucoagglutinin*; SL, serrated lesion; SPL, serrated pathway lesion; SSL, sessile serrated lesion; *r*, correlation coefficient; RT-PCR, reverse transcriptase-quantitative polymerase chain reaction. *Correlation coefficient.

benefit from a shorter interval of follow-up, to reduce interval cancers. However, we did not distinguish the dysplasia type of CA (high-grade vs. low-grade dysplasia), which may limit these conclusions.

According to our data, smoking and synchronous CA presence seem to be risk factors for progression to CRC in the serrated pathway, since they are correlated with high-mannose *N*-glycans downregulation on T cells, suggesting decreased immune function in SPL. Furthermore, it seems that the lower immune system activation by downregulation of high-mannose *N*-glycans enables the synchronous CA development. In fact, Li et al. [21] verified that the synchronous CA presence in patients with SL confers an increased risk of developing CRC. Regarding this, our results are in accordance with the literature, emphasizing *N*-glycosylation as a new potential biomarker for malignancy progression in serrated pathway. Contrariwise, we also showed an increased stromal high-mannose *N*-glycans expression in the presence of dysplasia and adenocarcinoma with concomitant SSL-D and in the left colon lesions. These findings suggest that the immune system is more active both in the presence of dysplasia and adenocarcinoma of the serrated pathway, contradicting what we expected. We predicted a reduced high-mannose *N*-glycans in immune cells related with T cells inability to recognize neoplastic lesions. Thereby, we need further investigations with a larger sample size to clarify these results. Regarding SPL location, left colon lesions appear to have a more active immunological profile, by having a higher high-mannose *N*-glycans expression, suggesting that this location is less likely to progress to CRC. In fact, SPL is more frequent in the right colon [22]. These results have a follow-up impact, considering the low potential for progression of a left colon lesion. Therefore, the *N*-glycosylation profile in colonic immune compartment could be used to guide clinicians on follow-up decision.

As previously mentioned, we found an association between the β1,6-GlcNAc branched *N*-glycans in epithelial component and a high BMI. An increased BMI was also associated with a reduced high-mannose *N*-glycans expression on Tregs. These results suggest that Tregs might have an increased immunosuppressive capacity in the presence of elevated BMI, creating an immunosuppressive microenvironment, which contributes to progression to malignancy. Thus, as emphasized earlier, patients with higher BMI may be considered for a closer surveillance of SPL.

Regarding gender, a different *N*-glycosylation pattern was observed in different immune system cells. In fact, β1,6-GlcNAc *N*-glycosylation in different genders has

never been studied before. Our results showed a higher high-mannose *N*-glycosylation expression in $CD4^+$ T cells in females and in $CD8^+$ T cells in males in all serrated pathway, highlighting that men and women have higher activation of different T cells. Both $CD8^+$ and $CD4^+$ T cells play a role in tumor eradication by direct action ($CD8^+$ T cells) and cytokine release ($CD4^+$ T cells) [12]. Despite this, $CD8^+$ T cells infiltration in tumor microenvironment is correlated with a better prognosis in CRC, suggesting that men have lower progression to cancer in serrated pathway [23]. By opposition, Tregs in females have a higher β1,6-GlcNAc branched *N*-glycans expression creating an immunosuppressive environment that allows the progression to malignancy. Regarding this, our results suggest a different *N*-glycosylation pattern of immune system cells occurring in both genders, which demands further investigation.

To conclude, biomarkers are an essential tool in current medical practice, playing an important role on understanding and identifying several diseases. Our study showed that *N*-glycosylation could be a potential biomarker of tumor progression in the serrated pathway. This study set the ground for the potential inclusion of β1,6-GlcNAc branched *N*-glycans and high-mannose *N*-glycans in the SPL anatomopathological analysis to select those patients who need shorter intervals of follow-up to reduce the interval cancers incidence. Furthermore, according to *N*-glycosylation profile, we identified smoking, aging, elevated BMI, SL dimensions ≥10 mm, the presence and number of synchronous CA as risk factors to progression to malignancy. These associations allow directed clinical interventions based on risk factors to reduce the progression to malignancy. Taken together, *N*-glycosylation profile seems to be one key to solve this puzzling pathway with large impact in clinical and molecular research. Despite our results, this study has several limitations, namely, the small sample size, the high missing data value, and the lack of similar articles that prevent us to draw more conclusions.

Statement of Ethics

This study protocol was reviewed and approved by Departamento de Ensino, Formação e Investigação (DEFI), and ethical committee of Centro Hospitalar Universitário de Santo António, number 2021.306 (252-DEFI/260-CE). Informed consent was not required, decided by ethical committee of Centro Hospitalar Universitário de Santo António.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This article had the funding support of Liga Portuguesa Contra o Cancro (LPCC) and MultiCare (Grant/Award No. C 3035006C17). This work was also funded by Raquel Seruca Porto Comprehensive Cancer Centre (P.CCC). Catarina M. Azevedo thanks Fundação para a Ciência e Tecnologia (FCT) for a fellowship (2021.07357.BD).

Author Contributions

Henrique Fernandes-Mendes: conceptualization; methodology; investigation; formal analysis; and writing – original draft; Catarina M. Azevedo: conceptualization; methodology; investigation; and writing – review and editing. Mónica Garrido: conceptualization and writing – review and editing. Carolina Lemos: methodology and formal analysis. Isabel Pedroto: supervision. Salomé S. Pinho: writing – review and editing and supervision. Ricardo Marcos-Pinto and Ângela Fernandes: conceptualization; methodology; investigation; formal analysis; writing – review and editing; and supervision. All authors approved the final version of the manuscript.

Data Availability Statement

This article was based on a final master's thesis of medical training, which will be published on Repositório da Universidade do Porto on June 28, 2023.

References

1 Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet. 2019; 394(10207):1467–80.
2 Roquette R, Painho M, Nunes B. Geographical patterns of the incidence and mortality of colorectal cancer in mainland Portugal municipalities (2007–2011). BMC Cancer. 2019;19(1):512.
3 Crockett SD, Nagtegaal ID. Terminology, molecular features, epidemiology, and management of serrated colorectal neoplasia. Gastroenterology. 2019;157(4):949–66.e4.
4 Kim JH, Kang GH. Evolving pathologic concepts of serrated lesions of the colorectum. J Pathol Transl Med. 2020;54(4): 276–89.
5 Bell PD, Anderson JC, Srivastava A. The frontiers of serrated polyps. Am J Surg Pathol. 2022;46(1):E64–70.
6 Okamoto K, Kitamura S, Kimura T, Nakagawa T, Sogabe M, Miyamoto H, et al. Clinicopathological characteristics of serrated polyps as precursors to colorectal cancer: current status and management. J Gastroenterol Hepatol. 2017;32(2):358–67.
7 Rashtak S, Rego R, Sweetser SR, Sinicrope FA. Sessile serrated polyps and colon cancer prevention. Cancer Prev Res. 2017;10(5):270–8.

8 Acosta-Gonzalez G, Ouseph M, Lombardo K, Lu S, Glickman J, Resnick MB. Immune environment in serrated lesions of the colon: intraepithelial lymphocyte density, PD-1, and PD-L1 expression correlate with serrated neoplasia pathway progression. Hum Pathol. 2019;83:115–23.
9 Murakami T, Sakamoto N, Nagahara A. Clinicopathological features, diagnosis, and treatment of sessile serrated adenoma/polyp with dysplasia/carcinoma. J Gastroenterol Hepatol. 2019;34(10):1685–95.
10 O'Connell BM, Crockett SD. The clinical impact of serrated colorectal polyps. Clin Epidemiol. 2017;9:113–25.
11 Pinho SS, Reis CA. Glycosylation in cancer: mechanisms and clinical implications. Nat Rev Cancer. 2015;15(9):540–55.
12 Fernandes Â, Azevedo CM, Silva MC, Faria G, Dantas CS, Vicente MM, et al. Glycans as shapers of tumour microenvironment: a sweet driver of T-cell-mediated anti-tumour immune response. Immunology. 2023;168(2):217–32.
13 Pereira MS, Alves I, Vicente M, Campar A, Silva MC, Padrão NA, et al. Glycans as key checkpoints of T cell activity and function. Front Immunol. 2018;9:2754.
14 Silva MC, Fernandes Â, Oliveira M, Resende C, Correia A, de-Freitas-Junior JC, et al. Glycans as immune checkpoints: removal of branched N-glycans enhances immune recognition preventing cancer progression. Cancer Immunol Res. 2020; 8(11):1407–25.
15 Demetriou M, Granovsky M, Quaggin S, Dennis JW. Negative regulation of T-cell activation and autoimmunity by Mgat5 N-glycosylation. Nature. 2001; 409(6821):733–9.
16 Dias AM, Correia A, Pereira MS, Almeida CR, Alves I, Pinto V, et al. Metabolic control of T cell immune response through glycans in inflammatory bowel disease. Proc Natl Acad Sci U S A. 2018;115(20):E4651–60.
17 Bouwens MWE, Winkens B, Rondagh EJA, Driessen AL, Riedl RG, Masclee AAM, et al. Simple clinical risk score identifies patients with serrated polyps in routine practice. Cancer Prev Res. 2013;6(8):855–63.
18 He X, Wu K, Ogino S, Giovannucci EL, Chan AT, Song M. Association between risk factors for colorectal cancer and risk of serrated polyps and conventional adenomas. Gastroenterology. 2018;155(2):355–73.e18.
19 Bailie L, Loughrey MB, Coleman HG. Lifestyle risk factors for serrated colorectal polyps: a systematic review and meta-analysis. Gastroenterology. 2017;152(1):92–104.
20 Hassan C, Antonelli G, Dumonceau JM, Regula J, Bretthauer M, Chaussade S, et al. Post-polypectomy colonoscopy surveillance: European society of gastrointestinal endoscopy (ESGE) guideline – update 2020. Endoscopy. 2020;52(8):687–700.
21 Li D, Doherty AR, Raju M, Liu L, Lei NY, Amsden LB, et al. Risk stratification for colorectal cancer in individuals with subtypes of serrated polyps. Gut. 2021:gutjnl-2021-324301.
22 Cassese G, Amendola A, Maione F, Giglio MC, Pagano G, Milone M, et al. Serrated lesions of the colon-rectum: a focus on new diagnostic tools and current management. Gastroenterol Res Pract. 2019;2019: 9179718.
23 Koch M, Beckhove P, Op Den Winkel J, Autenrieth D, Wagner P, Nummer D, et al. Tumor infiltrating T lymphocytes in colorectal cancer: tumor-selective activation and cytotoxic activity in situ. Ann Surg. 2006; 244(6):986–92; discussion 992–3.
24 Macarie M, Bățagă S, Mocan S, Pantea M, Opaschi R, Voidazan S, et al. Correlation of metabolic risk factors with sessile serrated lesions. J Gastrointest Liver Dis. 2020;29(2): 175–9.
25 Anwar S, Cock C, Young J, Young GP, Meng R, Simpson K, et al. Features associated with high-risk sessile serrated polyps at index and follow-up colonoscopy. J Gastroenterol Hepatol. 2021;36:1620–6.
26 Symonds E, Anwar S, Young G, Meng R, Coats M, Simpson K, et al. Sessile serrated polyps with synchronous conventional adenomas increase risk of future advanced neoplasia. Dig Dis Sci. 2019;64(6):1680–5.

GE – Portuguese Journal of Gastroenterology

Research Article

GE Port J Gastroenterol 2024;31:351–359
DOI: 10.1159/000536216

Received: May 19, 2023
Accepted: December 28, 2023
Published online: February 6, 2024

Long-Term Follow-Up of Kidney Function after Acute Liver Failure or Acute Liver Injury: A Cohort Study

Pedro Fidalgo[a] Pedro Póvoa[a, b] Nuno Germano[c] Constantine J. Karvellas[d] Filipe S. Cardoso[c, d, e]

[a]Polyvalent Intensive Care Unit, São Francisco Xavier Hospital, Centro Hospitalar Lisboa Ocidental, Lisbon, Portugal; [b]Nova Medical School, Nova University of Lisbon, CHRC, CEDOC, Lisbon, Portugal; [c]Intensive Care Unit, Curry Cabral Hospital, Lisbon, Portugal; [d]Division of Gastroenterology (Liver Unit), Department of Critical Care Medicine University of Alberta, Edmonton, AB, Canada; [e]Transplant Unit, Intensive Care Unit, Curry Cabral Hospital, Nova Medical School, Nova University, Lisbon, Portugal

Keywords
Liver failure · Renal insufficiency · Chronic kidney disease

Abstract

Introduction: Acute liver failure (ALF) is a rare disease with high mortality. Acute kidney injury (AKI) following ALF is frequent. We assessed AKI impact on long-term kidney function among ALF survivors. ***Methods:*** Observational cohort study including consecutive adult (age ≥16 years) patients with ALF or acute liver injury (ALI) admitted to a Portuguese tertiary center intensive care unit (ICU) between October 2013 and February 2020. KDIGO criteria were used to define AKI and chronic kidney disease (CKD). Primary outcome was the estimated glomerular filtration rate (eGFR), defined by the Chronic Kidney Disease Epidemiology Collaboration formula, at least 1 year after index ICU admission. ***Results:*** Among 104 patients with ALF (*n* = 74) or ALI (*n* = 30), mean (SD) age was 43.7 (18.0) years, and 44 were male. Among all patients (*n* = 104), following adjustment for age and SOFA score, AKI during the first 7 ICU days (*n* AKI = 57 and *n* renal replacement therapy [RRT] = 32) was independently associated with all-cause mortality (adjusted HR [95% CI] 11.61 [1.49–90.34]; *p* = 0.019). Among hospital survivors with long-term kidney function available (*n* = 56), median (interquartile range) >1 year eGFR was 95.3 (75.0–107.7) mL/min/1.73 m^2 (mean [SD] follow-up of 3.1 [1.6] years). Among these hospital survivors, following adjustment for baseline eGFR, AKI during the first 7 ICU days (*n* AKI = 19 and *n* RRT = 10) was not associated with >1 year eGFR (*p* = 0.15). At least 1 year after index ICU admission, 5 patients developed CKD, none RRT-dependent. ***Conclusions:*** Among ALF or ALI survivors, AKI was not associated with significant long-term loss of kidney function.

Função renal de seguimento a longo-prazo após falência hepática aguda ou lesão hepática aguda: um estudo coorte

Palavras Chave
Falência hepática · Insuficiência renal · Doença renal crónica

karger@karger.com
www.karger.com/pjg

Correspondence to:
Filipe S. Cardoso, filipe_sousacardoso@hotmail.com

Resumo

Introdução: A falência hepática aguda (ALF) é uma doença rara com alta mortalidade. A lesão renal aguda (AKI) após ALF é frequente. Avaliamos o impacto da AKI na função renal de longo prazo entre os sobreviventes de ALF. ***Métodos:*** Estudo observacional de coorte incluindo adultos consecutivos (idade ≥16 anos) com FHA ou lesão hepática aguda (ALI) internados numa unidade de cuidados intensivos (UCI) num centro terciário português entre Outubro de 2013 e Fevereiro de 2020. Os critérios KDIGO foram usados para definir AKI e doença renal crónica (CKD). O endpoint primário foi a taxa de filtração glomerular estimada (eGFR), definida pela fórmula da Chronic Kidney Disease Epidemiology Collaboration, pelo menos um ano após a admissão na UCI. ***Resultados:*** Entre 104 pacientes com ALF (*n* = 74) ou ALI (*n* = 30), a idade média (DP) foi de 43.7 (18.0) anos e 44 eram do sexo masculino. Entre todos os pacientes (*n* = 104), após ajuste para idade e score SOFA, AKI durante os primeiros 7 dias de UCI (*n* AKI = 57 e n terapia de substituição renal (RRT) = 32) foi independentemente associada à mortalidade por todas as causas (HR ajustado [IC 95%] 11.61 [1.49–90.34]; *p* = 0.019). Entre os sobreviventes no hospital com função renal de longo prazo disponível (*n* = 56), a eGFR mediana (IQR) >1 ano foi de 95.3 (75.0–107.7) mL/min/1.73 m^2 (média [DP] de acompanhamento de 3.1 [1.6] anos). Entre esses sobreviventes, após ajuste para eGFR basal, AKI durante os primeiros 7 dias de UCI (*n* AKI = 19 e n RRT = 10) não se associou com a eGFR >1 ano (*p* = 0.15). Pelo menos 1 ano após admissão na UCI, 5 pacientes desenvolveram DRC, nenhum dependente de RRT. ***Conclusões:*** Entre os sobreviventes de ALF ou ALI, AKI não se associou com perda significativa da função renal a longo prazo.

Introduction

Acute kidney injury (AKI) is a frequent complication among patients with acute liver failure (ALF), occurring in >50% of patients, and has been associated with poorer short- and long-term clinical outcomes [1–3]. Higher serum creatinine (sCr) has been associated with lower transplant-free survival in paracetamol overdose (APAP) patients; therefore, sCr is one of the prognostic criteria used to select patients for emergency orthotopic liver transplant (OLT) [4, 5].

The association between AKI and the development of chronic kidney disease (CKD) has been extensively reported among survivors of critical illness, and a relationship between the severity of AKI and the magnitude of CKD risk has been consistently described [6, 7].

In ALF, AKI or renal replacement therapy (RRT) at that time of OLT has not been associated with increased risk of CKD [3, 8]. However, data about the potential impact of AKI on long-term kidney function among ALF patients remain scarce. Specifically, little is known about the long-term kidney function among ALF survivors not submitted to OLT [9].

Accordingly, we hypothesized that AKI following ALF could have an impact on long-term kidney function. Therefore, the objectives of this study were the following: (1) to assess long-term kidney function in patients admitted to the intensive care unit (ICU) due to ALF or acute liver injury (ALI); (2) to evaluate the modifying impact of AKI or RRT on long-term kidney function in these patients.

Methods

Study Design, Setting, and Participants

We used a cohort from Curry Cabral Hospital (CCH), Central Lisbon University Hospital Center (CLUHC), prospective registry including all consecutive adult (age ≥16 years) patients with ALF or ALI admitted to the ICU between October 2013 and February 2020 (Fig. 1). We excluded patients with cirrhosis, previous OLT, or CKD under chronic RRT prior to ICU admission.

The liver transplant program started in 1992 is currently the largest in Portugal, performing 100–120 liver transplants per year. CCH is the referral center for all liver transplants in the country's south (catchment population of up to 3 million people). The Local Ethics Committee at CCH, CLUHC, has approved the study's protocol, and the need for informed consent was waived due to the observational nature of this study (INV_363).

All research procedures were conducted according to the principles of the Declaration of Helsinki [10]. The reporting of this study followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [11].

Operational Definitions

ALF was defined using the following criteria: (a) hepatic encephalopathy (HE) of any degree (West Haven criteria), (b) INR ≥1.5, (c) acute illness onset <26 weeks, and (d) no evidence of cirrhosis [1, 12]. ALI was defined as new liver dysfunction expressed as elevated serum transaminases (>3 times from the upper limit of normal) coupled with any degree of impaired liver function (INR or bilirubin) without concomitant HE or cirrhosis [1, 12]. AKI was diagnosed, and its severity was staged according to the sCr criteria of the KDIGO classification [13]. We defined the presence of AKI by an absolute increase in sCr ≥0.3 mg/dL or ≥1.5 fold relative change from baseline sCr in the first 7 days of ICU stay. Severity was classified as: stage 1, increase in sCr ≥0.3 md/dL or 1.5–1.9 times from baseline; stage 2, increase in sCr 2.0–2.9 times from baseline; and stage 3: increase in sCr 3.0 times from baseline, or an increase to

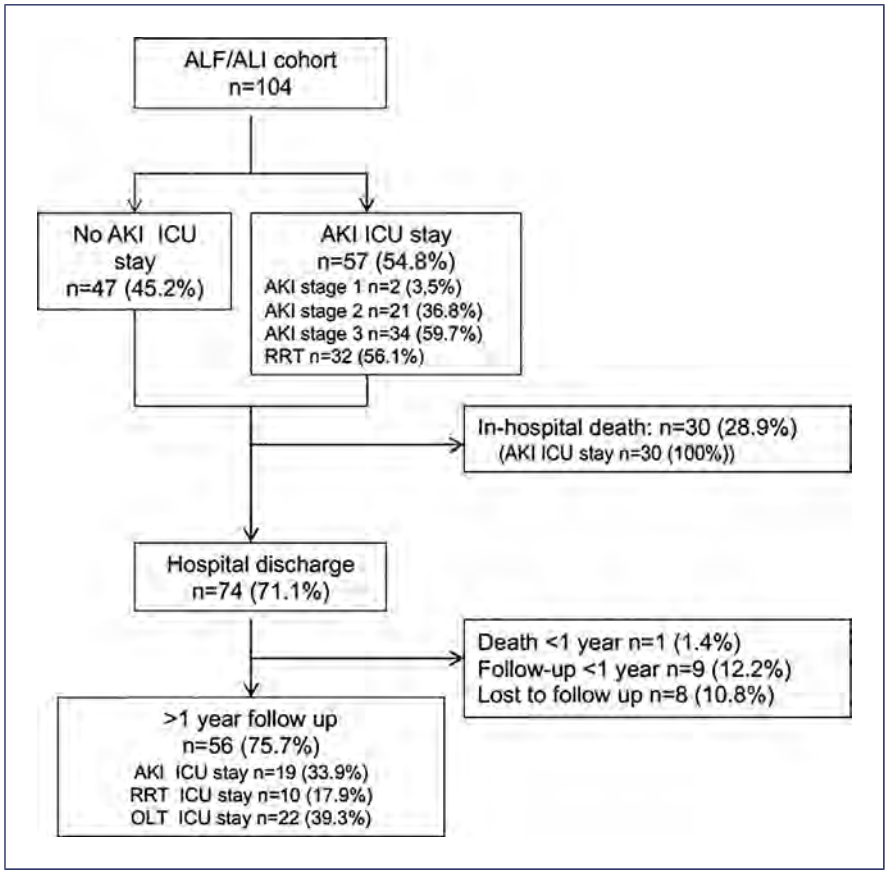


Fig. 1. Flowchart of the entire study cohort.

sCr ≥4.0 mg/dL or RRT initiation. The use of RRT was not standardized; therefore, indications, modality, treatment dose, anticoagulation, and criteria for initiation and suspension of the technique were based on individual clinical judgment. Any patient on RRT was considered to have AKI even if the technique was started for a non-kidney reason (e.g., clearance of ammonia or toxins or temperature control), as sCr loses its diagnostic value under RRT [1]. Urine output data were not consistently available.

Baseline sCr was defined as the lowest sCr value available prior to the day of hospital admission and, if not available, was calculated according to the KDIGO recommendations [14]. Baseline estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula. CKD was defined as eGFR <60 mL/min/1.73 m^2 according to the KDIGO definition [14].

Long-term kidney function was defined as one determination of sCr in steady state >1 year after index ICU admission. The most recent sCr available after >1-year follow-up was considered. Those patients not being followed in CLUHC outpatient clinic were contacted via telephone and asked to provide the most recent laboratory data to investigators.

Exposures and Endpoints

The ALF registry at CCH data captures demographic, clinical, and laboratory data during the first 7 days of ICU stay including: age, sex, and etiology; HE grade (West Haven criteria), invasive mechanical ventilation use, PaO_2/FiO_2 ratio (mm Hg), vasopressor use, mean arterial pressure (mm Hg), RRT use, and Sequential Organ Failure Assessment (SOFA) score; laboratory serum profile including INR, bilirubin (mg/dL), alanine aminotransferase (U/L), ammonia (μmol/L), Factor V (%), creatinine (mg/dL), bicarbonate (mmol/L), pH, lactate (mmol/L), hemoglobin (g/dL), and platelet count (10^3 cells/μL); and immunosuppression regimen [15]. Analysis 1 included all patients with ALF or ALI admitted to the ICU (n = 104) and assessed the impact of AKI or RRT during the first 7 days of index ICU stay (exposures) on all-cause mortality (endpoint) (Fig. 1). Analysis 2 considered only hospital survivors (n = 74) and evaluated the impact of AKI or RRT during the first 7 days of index ICU stay (exposures) on eGFR >1 year after index ICU admission (endpoint).

Statistical Analysis

Descriptive statistics were calculated and expressed as mean (standard deviation [SD]) or median (interquartile range [IQR]) for parametric and non-parametric continuous variables, respectively, and count (%) for categorical variables. Univariable comparisons were performed with χ^2, Fisher's, Student's t, Mann-Whitney, Wilcoxon, or Kruskal-Wallis tests where appropriate. Missing data across all values were 8.4%, and no multiple imputation was performed.

In analysis one, survival analysis with Kaplan-Meier curves (log-rank test) and multivariable Cox proportional hazard model was performed to examine the association of covariables with all-cause

mortality. In analysis 2, multivariable linear regression was used to describe the association of covariables with >1-year eGFR. Covariables initially considered for modeling were those with a p value <0.10 on univariable comparisons. A backward stepwise selection process was performed to select final models' composition based on the best models' performance while avoiding overfitting. Covariables were assessed for multicollinearity and excluded accordingly. Models' performance was assessed by the χ^2 or R^2 statistics.

A p value <0.05 (2-tailed) was considered statistically significant for all comparisons. Statistical analysis was performed using SPSS version 21.0 (IBM Corp., Armonk, NY, USA).

Results

Patients' Baseline Characteristics

Among 104 patients included, 74 (71.2%) had ALF, and 30 (28.8%) had ALI. Mean (SD) age was 43.7 (18.0) years, and 44 (42.3%) were males (Fig. 1). The main known causes of ALF/ALI were the following: paracetamol toxicity in 18 patients (17.3%), other drug-induced liver injury in 18 (17.3%), viral hepatitis in 11 (10.6%), and ischemia in 8 (7.7%) (Table 1; online suppl. Table S1; for all online suppl. material, see https://doi.org/10.1159/000536261). Only 3 (2.9%) patients had baseline CKD.

On ICU day one, mean (SD) SOFA score was 7.6 (4.8). Overall, 27 (26.0%) patients had grade 3–4 HE, 21 (20.2%) were mechanically ventilated, 19 (18.3%) were on vasopressors, and 43 (41.3%) had AKI, of which 15 (14.4%) required RRT. At this time point, median (IQR) serum INR, bilirubin, creatinine, and lactate were 2.5 (1.8–3.9), 9.1 (2.7–19.2) mg/dL, 0.86 (0.66–1.55) mg/dL, and 2.0 (1.5–3.4) mmol/L, respectively (Table 1). All baseline characteristics are depicted in Table 1.

Outcomes

Overall, during a mean (SD) follow-up time of 2.4 (1.9) years, 34 (32.7%) patients required OLT and 32 (30.8%) died, 30 (93.8%) of these during the index hospital stay (Table 1; online suppl. Fig. S2). Among the deceased patients, 24 (75%) died without OLT. The main cause of death was ALF-related multiorgan failure in 46.9% (15/32) of cases (online suppl. Fig. S3).

Median (IQR) time to OLT and death were 3 (1–6) and 5 (3–11) days, respectively. Median ICU and hospital length-of-stay were 5 (3–10) and 19 (8–34) days, respectively.

Analysis One: Associations with All-Cause Overall Mortality

Among 104 patients included, during the first 7 days of index ICU stay, 57 (54.8%) had AKI (43 diagnosed on ICU day one and 14 over the following 6 days): stage 1 in 3.5% (n = 2), stage 2 in 36.8% (n = 21), and stage 3 in 59.7% (n = 34). RRT was required in 56.1% of those with AKI (32/57, with 15 started on ICU day one). Continuous RRT (either venovenous hemodiafiltration or hemofiltration) was the modality most frequently used (71.9% [23/32] of all RRT prescriptions).

AKI patients were older (mean age of 47.7 vs. 38.9 years; p = 0.01) and had more often ALF (82.5% vs. 57.4%; p = 0.005) than others. Furthermore, on ICU day one, AKI patients had higher proportion of mechanical ventilation support (31.6% vs. 6.4%; p = 0.001), with lower mean PaO_2/FiO_2 ratio (341.8 vs. 428.7 mm Hg; p < 0.001), higher proportion of vasopressor use (28.1% vs. 6.4%; p = 0.004), higher median lactate (2.9 vs. 1.9 mmol/L; p < 0.001), and higher overall disease severity (mean SOFA score of 10 vs. 5; p < 0.001) than those without AKI. On ICU day one, ALF patients had higher overall disease severity (mean SOFA score of 8 vs. 3; p < 0.001) and were more frequently diagnosed with AKI (63.5% vs. 33.3%; p = 0.005) in comparison to those with ALI.

On univariable analysis, AKI patients or those under RRT had higher all-cause mortality than others (online suppl. Fig. S4). However, among AKI patients, AKI staging proportions (1 or 2 vs. 3) were similar between survivors and non-survivors (46.2% vs. 35.5%; p = 0.47). On multivariable analysis with Cox regression, age {adjusted hazard ratio (aHR) (95% confidence interval [CI]) of 1.03 (1.01–1.05); p = 0.014}, SOFA score on ICU day one (aHR [95% CI] of 1.25 [1.14–1.36]; p < 0.001), and AKI on the first 7 days of ICU stay (aHR [95% CI] of 11.39 [1.46–88.91]; p = 0.019) were independently associated with higher hazard of all-cause mortality (Table 2). A similar effect was observed in a sensitivity analysis following exclusion of ALI patients (online suppl. Table S1).

Analysis 2: Associations with >1-Year eGFR

Among 74 patients discharged alive from the hospital, 27 (36.5%) had AKI during the first 7 days of the index ICU stay, with 13 (17.6%) requiring RRT. Only 56 (75.7%) of these patients had available >1-year kidney function assessment and were considered for this analysis (Fig. 1). Among these 56 hospital survivors, 19 (33.9%) had AKI during the first 7 days of ICU stay, with 10 (17.9%) requiring RRT. AKI and RRT proportions were thus similar between the total number of hospital survivors (n = 74) and the subgroup of those with long-term kidney function assessment available (n = 56) (online suppl. Table S2).

Among these 56 patients, median (IQR) >1-year eGFR was 95.3 (75.0–107.7) mL/min/1.73 m^2 for a mean (SD)

Table 1. Patients' baseline characteristics on ICU day one and outcomes stratified by AKI status on the first 7 days of stay (n = 104)

Characteristics	Overall 104 (100)	AKI 7 days ICU 57 (54.8)	No AKI 7 days ICU 47 (45.2)	p value
Demographic				
Age, years	43.7 (18.0)	47.7 (20.7)	38.9 (12.6)	0.013
Sex (male)	44 (42.3)	28 (49.1)	16 (34.0)	0.12
Etiology (APAP vs. other)	18 (17.3)	9 (15.8)	9 (19.1)	0.65
ALF (vs. ALI)	74 (71.2)	47 (82.5)	27 (57.4)	0.005
Baseline eGFR, mL/min/ 1.73 m^2	79.3 (76.7–80.6)	78.9 (73.8–80.3)	79.6 (78.3–89.1)	0.003
Baseline CKD	3 (2.9%)	3 (5.3%)	0 (0%)	0.25
Organ failures and support				
HE grade 3–4 (vs. other)	27 (26.0)	19 (33.3)	8 (17.0)	0.06
Mechanical ventilation	21 (20.2)	18 (31.6)	3 (6.4)	0.001
PaO_2/FiO_2 ratio, mm Hg	381.1 (126.3)	341.8 (128.9)	428.7 (106.1)	<0.001
Vasopressor use	19 (18.3)	16 (28.1)	3 (6.4)	0.004
MAP, mm Hg	81.9 (69.3–89.8)	79.2 (66.4–90.4)	82.2 (75.2–89.1)	0.19
SOFA score	8 (5)	10 (5)	5 (4)	<0.001
Laboratory parameters				
INR	2.5 (1.8–3.9)	2.4 (2.0–3.9)	2.7 (1.8–4.8)	0.045
Bilirubin, mg/dL	9.1 (2.7–19.2)	5.2 (3.2–18.8)	15.1 (6.1–21.7)	0.24
ALT, U/L, n = 103	1,475 (252–4,086)	2,736 (345–5,800)	1,445 (353–2,431)	0.68
Ammonia, µmol/L, n = 78	133 (83–189)	163 (88–270)	123 (79–189)	0.017
Factor V (%, n = 72)	42.5 (17.3–69.8)	22.0 (14.0–42.0)	65.5 (44.3–82.0)	0.001
Creatinine, mg/dL	0.86 (0.66–1.55)	1.57 (1.10–2.19)	0.61 (0.50–0.72)	<0.001
Urea, mg/dL	28.0 (15.0–65.0)	65.0 (36.0–120.0)	15.0 (11.3–17.8)	<0.001
Lactate, mmol/L, n = 93	2.0 (1.5–3.4)	2.9 (1.8–6.8)	1.9 (1.2–2.3)	<0.001
HCO_{3-}, mmol/L, n = 94	22.0 (18.5–25.0)	20.0 (16.0–23.0)	24.0 (21.3–24.9)	<0.001
pH (n = 94)	7.43 (7.37–7.48)	7.39 (7.32–7.45)	7.47 (7.44–7.49)	<0.001
Hemoglobin, g/L	123 (107–135)	130 (116–143)	126 (115–134)	0.54
Platelets, 10^3 cells/µL	146 (68–239)	108 (58–244)	156 (110–238)	0.027
Outcomes, n (%)				
OLT	34 (32.7)	18 (31.2)	16 (34.0)	0.79
Death	32 (30.8)	31 (54.4)	1 (2.1)	<0.001

Results are presented as n (%), mean (SD), or median (IQR). AKI, acute kidney injury; ICU, intensive care unit; SD, standard deviation; IQR, interquartile range; APAP, paracetamol overdose; HE, hepatic encephalopathy; ALF, acute liver failure; ALI, acute liver injury; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; MAP, mean arterial pressure; SOFA, sequential organ failure assessment; INR, international normalized ratio; ALT, alanine transferase; OLT, orthotopic liver transplant.

Table 2. Cox regression model: multivariable analysis of the association between covariables and overall all-cause mortality among all patients included (n = 104)

Variables	HR	95% CI		p value
		lower	upper	
Age (years)	1.03	1.01	1.05	0.014
SOFA score (ICU day one) (0–24)	1.24	1.14	1.36	<0.001
AKI (7 days of ICU stay)	11.61	1.49	90.34	0.019

Model: n = 104, n events of death = 32; χ^2 statistic = 66, $p < 0.001$. HR, hazard ratio; confidence interval; SOFA, sequential organ failure assessment; AKI, acute kidney injury; ICU, intensive care unit.

follow-up time of 3.1 (1.6) years. Among the 56 hospital survivors, 8.9% (n = 5) developed CKD, none RRT-dependent. CKD prevalence among hospital survivors was similar between those who had AKI or not (10.5% vs. 8.1%, p = 1.0) or those that required RRT or not (10.0% vs. 8.7%; p = 1.0) during the index ICU stay. Among these

56 patients, median (IQR) >1 year eGFR was significantly higher than baseline eGFR (79.3 [76.7–80.6] versus 95.3 [75.0–107.7] mL/min/1.73 m^2; $p = 0.023$).

Among 36 patients transplanted, 21 had available data on calcineurin inhibitor use: 20 were on tacrolimus and 1 was on cyclosporin. For those on tacrolimus, latest single time point within >1 year follow-up mean (SD) through levels was 6.5 (2.2) ng/mL. Among the 56 hospital survivors, median >1 year eGFR was similar between those who had AKI or not (93.0 vs. 96.8 mL/min/1.73 m^2; $p = 0.76$), were treated with RRT or not (102.3 vs. 94.2 mL/min/1.73 m^2; $p = 0.32$), who underwent OLT or not (93.2 vs. 97.1 mL/min/1.73 m^2; $p = 0.21$), or who had ALF versus ALI (93.2 vs. 99.4 mL/min/1.73 m^2; $p = 0.54$) during the index ICU stay (Fig. 2).

On multivariable analysis with liner regression, while higher baseline eGFR was found to be independently associated with higher >1 year eGFR ($p < 0.001$), AKI during the first 7 days of the index ICU stay ($p = 0.75$) was not associated with long-term eGFR (Table 3). The linear regression equation was the following: >1 year eGFR = −398 + 6.22* baseline eGFR + 7.57* AKI (7 days) (baseline eGFR in mL/min/1.73 m^2, AKI [7 days] 0 if no or 1 if yes). A similar effect was observed in a sensitivity analysis following exclusion of ALI survivors (online suppl. Table S3).

Discussion

Key Results and Comparisons with Previous Literature

To the best of our knowledge, we presented a cohort of ALF or ALI patients with one of the longest follow-up evaluation of kidney function. Among all ALF or ALI patients, AKI occurrence was associated with worse all-cause mortality. Among ALF or ALI survivors, long-term kidney function was largely preserved with a median (IQR) >1 year eGFR of 95.3 (75.0–107.7) mL/min/1.73 m^2 irrespective of AKI diagnosis, RRT use, OLT need, or ALF versus ALI occurrence during the index ICU stay.

Contrary to our results, a previous study by Hadem et al. [16], including 134 ALF patients, found that sCr was significantly higher on follow-up after ICU admission. Differences between this study and ours might reflect their shorter follow-up period (median of 226 days following ICU admission) and their use of sCr rather than eGFR as method to quantify kidney function. Interestingly, in the study by Hadem et al. [16], the median follow-up sCr was 0.85 mg/dL, which in fact translates into an eGFR within normal range, considering the median age of their population, thus possibly also in agreement with our findings. Since the accuracy of eGFR as a surrogate for kidney function is poorer at higher levels of actual GFR, both studies suggest that ALF seems to have a modest impact on long-term kidney function. In fact, rates of CKD were close between the 2 cohorts (11.9% in theirs vs. 8.9% in ours). Furthermore, the increase in eGFR from baseline to >1 year we described probably does not translate into a clinically meaningful improvement in kidney function [17].

We also presented conflicting results with respect to the modifying impact of AKI or OLT post-ALF on the long-term kidney function. While Hadem et al. [16] found a significantly higher follow-up sCr among ALF patients that had AKI or OLT, we and others found that neither the occurrence of AKI nor the OLT per se significantly impacted long-term kidney function [3, 9, 16]. We recognize that OLT might lead to a second kidney insult, with new AKI episodes, whether due to surgical complications or organ-related ischemia-reperfusion injury. However, Leithead et al. [18] found that, among patients who underwent OLT due to ALF, AKI or the use of RRT at the time of transplant were not associated with increased risk of CKD [9, 18].

In ALF patients, kidney dysfunction has been described to improve more often than in chronic liver disease patients following OLT [19]. O'Riordan et al. [3] showed that, among patients with APAP and AKI that survived without OLT, 51% of patients returned to normal kidney function at the time of discharge (median follow-up of 38 days), and complete recovery (eGFR >60 mL/min/1.73 m^2) was observed in all of those followed for at least 3 months. The high recovery rates of AKI in the context of APAP may reflect the easily reversible nature of this agents' direct toxicity, both in the liver and in the kidneys [5, 20]. Besides direct toxicity to kidney cells related to specific ALF etiologies, such as mushroom poisoning, acetaminophen, and cotrimoxazole toxicity, or heat stroke-associated rhabdomyolysis, further etiology-independent injury mechanisms may be at play. While hypotension and systemic vasodilatation may contribute to reduction in renal blood flow, the few studies of renal blood flow in ALF are limited to animal models and show conflicting results [3, 18]. Alterations of the circulating concentration of vasoactive compounds, inflammation-associated cytokines, and damage-associated molecular patterns have all been implicated in AKI in ALF [5, 21]. Finally, kidney biopsies in patients with ALF have found focal tubular cell necrosis and focal vascular injury, predominantly of the

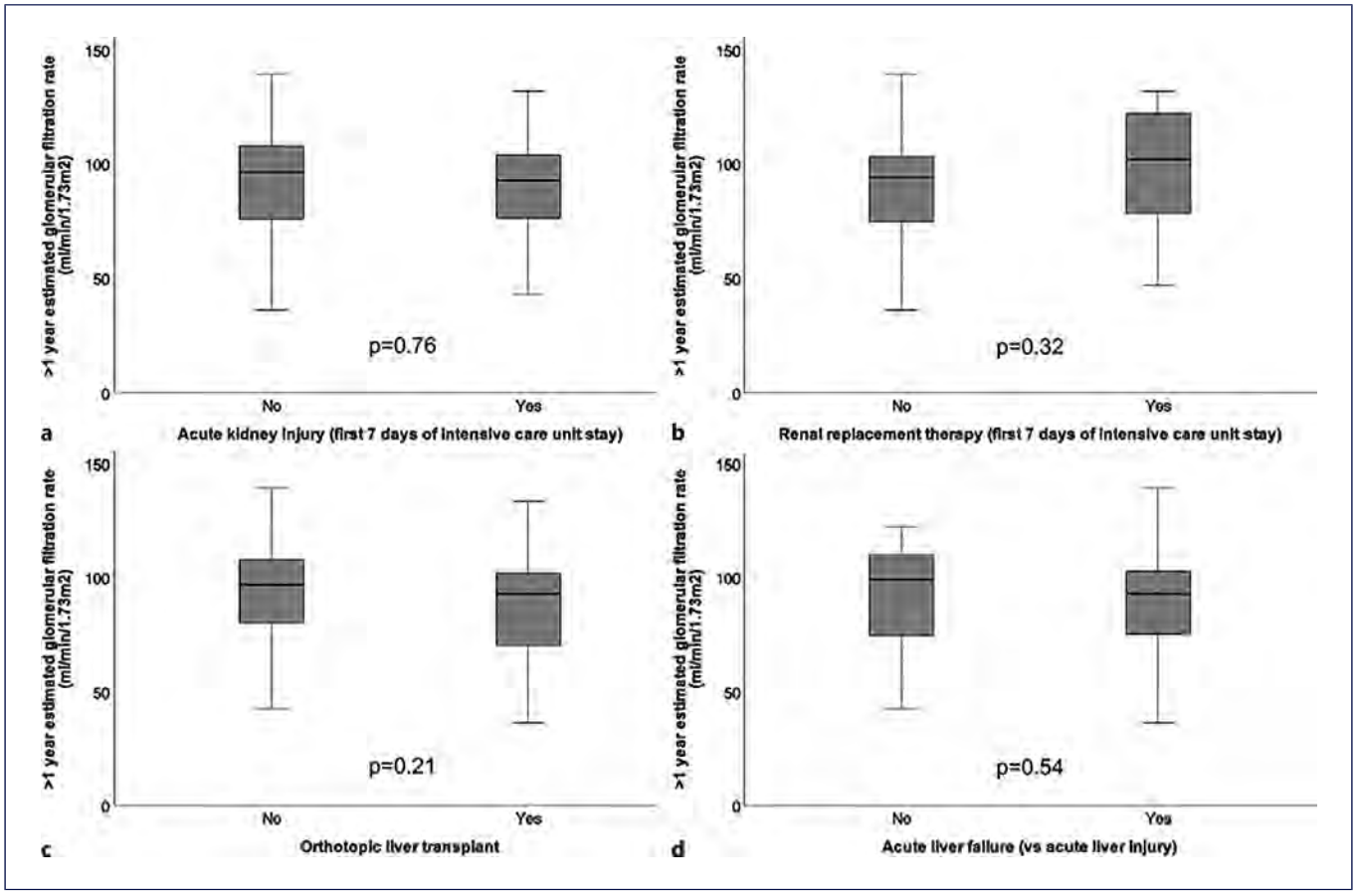


Fig. 2. Long-term eGFR (mL/min/1.73 m^2) stratified by AKI (**a**), RRT (**b**), OLT (**c**), and ALF versus ALI (**d**) status on index ICU stay among hospital survivors (n = 56).

Table 3. Linear regression model: multivariable analysis of the association between covariables and post 1-year eGFR among hospital survivors (n = 56)

Variables	β	95% CI		p value
		lower	upper	
Baseline eGFR, mL/min/1.73 m^2	6.22	3.80	8.64	<0.001
AKI (7 days of ICU stay)	7.57	−4.09	19.23	0.15

SE, standard error; CI, confidence interval; eGFR, estimated glomerular filtration rate; AKI, acute kidney injury; ICU, intensive care unit. Model: n included = 56. Regression equation: >1-year eGFR = −398 + 6.22 baseline eGFR +7.57 AKI (7 days) (baseline eGFR in mL/min/1.73 m^2, AKI [7 days] 0 if no or 1 if yes). Performance: R^2 0.34, p < 0.001.

endothelial cells in the glomeruli, peritubular capillaries, and small arterioles [22].

Moreover, the fact that ALF patients tend to be younger (median age <45 years) than the average critically ill patients with AKI, thus with lower number of comorbidities (<3% had baseline CKD), may further contribute to the higher rates of AKI recovery and the lower rates of distant CKD reported among these patients. This may help explain why AKI in ALF seems to have a lower long-term impact on kidney function in comparison to septic or other subgroups of critically ill patients.

Strengths, Limitations, and Implications

Some of the sicker ALF patients develop a severe systemic inflammatory response syndrome within the first few days of their disease course, often leading to distributive shock. Thus, ALF is likely most severe within the first 7 days of its course. The fact that we considered AKI diagnosis and RRT use for the first 7 days of the index ICU stay may have allowed to better capture the degree of kidney dysfunction that may ensue in the context of ALF.

Following ALF, the native liver may take months to regenerate. After OLT, nephrotoxic immunosuppression drugs may also take months to wean or adjust. In this context, the assessment of long-term kidney function >1 year following the index ICU admission (median follow-up time of 3 years) may have helped to better characterize the long-term impact of AKI in ALF or ALI survivors.

However, our results should be interpreted considering the following limitations. First, this was a single-center cohort, therefore prone to selection bias. Nevertheless, the local prospective registry including consecutive patients with standardized definitions and management approach may have mitigated such bias. Second, we did not have data on urine output to further improve the diagnostic evaluation of AKI, as recommended by the guidelines [13]. Nevertheless, sCr has been the most widely used biomarker for the assessment of kidney function in critically ill patients, and previous studies did not find added value of incorporating urinary output data to diagnose AKI in ALF [23, 24]. Finally, the absence of data on urinary sediment or biomarkers may have precluded any analyses considering possible different kidney injury mechanisms associated with diverse ALF etiologies, especially in a setting with a lower prevalence of APAP [25, 26].

Despite these limitations, we think our study adds to the literature by reinforcing that AKI may not negatively impact long-term kidney function in ALF or ALI survivors, a rather unique finding in the critical illness literature. In this context, strategies for kidney function surveillance following an AKI episode in ALF patients may be adapted accordingly. In the future, further studies could address gaps in knowledge about this topic, for example, by extending studies on the different mechanisms of kidney injury associated with diverse ALF etiologies.

Conclusions

Among ALF or ALI survivors, AKI during the first 7 days of ICU stay was not associated with significant loss of kidney function following at least 1 year of follow-up.

Statement of Ethics

This study protocol was reviewed and approved by the Central Lisbon University Hospital Center Ethics Committee (INV_363). The informed consent was waived by the Central Lisbon University Hospital Center Ethics Committee.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

None.

Author Contributions

Pedro Fidalgo and Filipe S. Cardoso conceived the idea, collected data, performed analysis, and wrote the manuscript. Pedro Póvoa, Nuno Germano, and Constantine J. Karvellas provided content expertise and approved the final version of the manuscript.

Data Availability Statement

Data may be available upon reasonable request.

References

1 European Association for the Study of the Liver Electronic address easloffice@easlofficeeu, Clinical practice guidelines panel, Wendon, J, Panel members; Cordoba J, Dhawan A. EASL Clinical Practical Guidelines on the management of acute (fulminant) liver failure. J Hepatol. 2017;66(5):1047–81.

2 O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology. 1989;97(2):439–45.

3 O'Riordan A, Brummell Z, Sizer E, Auzinger G, Heaton N, O'Grady JG, et al. Acute kidney injury in patients admitted to a liver intensive therapy unit with paracetamol-induced hepatotoxicity. Nephrol Dial Transplant. 2011;26(11):3501–8.

4 Pakravan N, Simpson KJ, Waring WS, Bates CM, Bateman DN. Renal injury at first presentation as a predictor for poor outcome in severe paracetamol poisoning referred to a liver transplant unit. Eur J Clin Pharmacol. 2009;65(2):163–8.

5 Lines SW, Wood A, Bellamy MC, Lewington AJP. The outcomes of critically ill patients with combined severe acute liver and kidney injury secondary to paracetamol toxicity requiring renal replacement therapy. Ren Fail. 2011;33(8):785–8.
6 See EJ, Jayasinghe K, Glassford N, Bailey M, Johnson DW, Polkinghorne KR, et al. Long-term risk of adverse outcomes after acute kidney injury: a systematic review and meta-analysis of cohort studies using consensus definitions of exposure. Kidney Int. 2019; 95(1):160–72.
7 Coca SG, Singanamala S, Parikh CR. Chronic kidney disease after acute kidney injury: a systematic review and meta-analysis. Kidney Int. 2012;81(5):442–8.
8 Leithead JA, Ferguson JW, Bates CM, Davidson JS, Simpson KJ, Hayes PC. Chronic kidney disease after liver transplantation for acute liver failure is not associated with perioperative renal dysfunction. Am J Transplant. 2011;11(9):1905–15.
9 Cardoso FS, Gottfried M, Tujios S, Olson JC, Karvellas CJ; US Acute Liver Failure Study Group. Continuous renal replacement therapy is associated with reduced serum ammonia levels and mortality in acute liver failure. Hepatology. 2018;67(2):711–20.
10 World Medical Association. World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. JAMA. 2013;310(20):2191–4.
11 von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. Lancet. 2007;370(9596):1453–7.
12 Cardoso FS, Marcelino P, Bagulho L, Karvellas CJ. Acute liver failure: an up-to-date approach. J Crit Care. 2017;39:25–30.
13 Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group. KDIGO clinical practice guideline for acute kidney injury. Kidney Int Suppl. 2012;2:1–138.
14 National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39(2 Suppl 1):S1–266.
15 Vincent JL, Moreno R, Takala J, Willatts S, De Mendonça A, Bruining H, et al. The SOFA (Sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the working group on sepsis-related problems of the European society of intensive care medicine. Intensive Care Med. 1996;22(7):707–10.
16 Hadem J, Kielstein JT, Manns MP, Kümpers P, Lukasz A. Outcomes of renal dysfunction in patients with acute liver failure. United Eur Gastroenterol J. 2019;7(3):388–96.
17 Poggio ED, Wang X, Greene T, Van Lente F, Hall PM. Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. J Am Soc Nephrol. 2005;16(2):459–66.
18 Leithead JA, Armstrong MJ, Corbett C, Andrew M, Kothari C, Gunson BK, et al. Hepatic ischemia reperfusion injury is associated with acute kidney injury following donation after brain death liver transplantation. Transpl Int. 2013;26(11):1116–25.
19 Aberg F, Koivusalo AM, Höckerstedt K, Isoniemi H. Renal dysfunction in liver transplant patients: comparing patients transplanted for liver tumor or acute or chronic disease. Transpl Int. 2007;20(7): 591–9.
20 Bagshaw SM, Laupland KB, Doig CJ, Mortis G, Fick GH, Mucenski M, et al. Prognosis for long-term survival and renal recovery in critically ill patients with severe acute renal failure: a population-based study. Crit Care. 2005;9(6):R700–09.
21 Larsen FS, Schmidt LE, Bernsmeier C, Rasmussen A, Isoniemi H, Patel VC, et al. High-volume plasma exchange in patients with acute liver failure: an open randomised controlled trial. J Hepatol. 2016;64(1):69–78.
22 Moore JK, Love E, Craig DG, Hayes PC, Simpson KJ. Acute kidney injury in acute liver failure: a review. Expert Rev Gastroenterol Hepatol. 2013;7(8):701–12.
23 Tujios SR, Hynan LS, Vazquez MA, Larson AM, Seremba E, Sanders CM, et al. Risk factors and outcomes of acute kidney injury in patients with acute liver failure. Clin Gastroenterol Hepatol. 2015;13(2):352–9.
24 Coelho S, Fonseca JN, Gameiro J, Jorge S, Velosa J, Lopes JA. Transient and persistent acute kidney injury in acute liver failure. J Nephrol. 2019;32(2):289–96.
25 Nadim MK, Genyk YS, Tokin C, Fieber J, Ananthapanyasut W, Ye W, et al. Impact of the etiology of acute kidney injury on outcomes following liver transplantation: acute tubular necrosis versus hepatorenal syndrome. Liver Transpl. 2012;18(5):539–48.
26 Levitsky J, Baker TB, Jie C, Ahya S, Levin M, Friedewald J, et al. Plasma protein biomarkers enhance the clinical prediction of kidney injury recovery in patients undergoing liver transplantation. Hepatology. 2014;60(6): 2017–26.

GE – Portuguese Journal of Gastroenterology

Endoscopic Snapshot

GE Port J Gastroenterol 2024;31:360–363
DOI: 10.1159/000535205

Received: August 4, 2023
Accepted: October 24, 2023
Published online: December 12, 2023

Anti-Reflux Mucosal Ablation: One More Kid in Town for the Treatment of Gastroesophageal Reflux Disease

Isabel Garrido[a, b] Armando Peixoto[a, b] Ana Luísa Santos[a, b] Rui Morais[a, b] Guilherme Macedo[a, b]

[a]Gastroenterology Department, Centro Hospitalar Universitário de São João, Porto, Portugal; [b]World Gastroenterology Organization (WGO) Porto Training Center, Porto, Portugal

Keywords
Anti-reflux mucosal ablation · Gastroesophageal reflux disease · Proton pump inhibitors

Ablação da mucosa anti-refluxo: uma nova opção no tratamento da doença de refluxo gastroesofágico

Palavras Chave
Ablação da mucosa anti-refluxo · Doença de refluxo gastroesofágica · Inibidores da bomba de protões

A 33-year-old man, with background of asthma, was referred to the gastroenterology outpatient clinic with daily heartburn and regurgitation over the last 2 years. No extra-esophageal reflux symptoms or dysphagia have been noted. Double-dose proton pump inhibitor (PPI) therapy for over 3 months was not successful. High-resolution esophageal manometry showed normal esophageal motility (Chicago Classification v4.0), normotensive lower esophageal sphincter (18.3 mm Hg), and normal integrated relaxation pressure (8.2 mm Hg). The 24-h pH-impedance test (off PPIs) revealed a pathological esophageal acid exposure time of 11.7% with a DeMeester score of 55.1. On impedance, the number of reflux episodes was 161, mainly acid refluxes, with positive symptomatic association (26 symptoms recorded by the patient). The Gastroesophageal Reflux Disease Health-Related Quality of Life Questionnaire (GERD-HRQL) score was 43 (ranges from 0–75) and the Frequency Scale for the Symptoms of GERD (FSSG) score was 35 (ranges from 0 to 48). Previous upper endoscopy showed grade B reflux esophagitis (Los Angeles classification) and grade II Hill's flap valve (2-cm hiatus hernia, no lesions in the hernial sac) (Fig. 1). Esophageal biopsies have been performed, excluding eosinophilic esophagitis. After discussing with the patient about therapeutic options, he was unwilling to undergo surgery; therefore, anti-reflux mucosal ablation (ARMA) was proposed.

ARMA was performed using the triangle-tip knife (Olympus®) connected to an electrocautery generator (VIO300D; ERBE Elektromedizin, Tübingen, Germany) in spray coagulation mode (50 W, effect 2) (online suppl. video; for all online suppl. material, see https://doi.org/10.1159/000535205). Mucosal ablation was performed around the cardia on the gastric side in a butterfly shape with a width of approximately 1.5 scope diameter, leaving two contralateral areas of normal cardia mucosa with approximately one scope diameter, to avoid stricture (Fig. 2). The procedure was done with propofol-based

karger@karger.com
www.karger.com/pjg

Correspondence to:
Isabel Garrido, isabelmng@hotmail.com

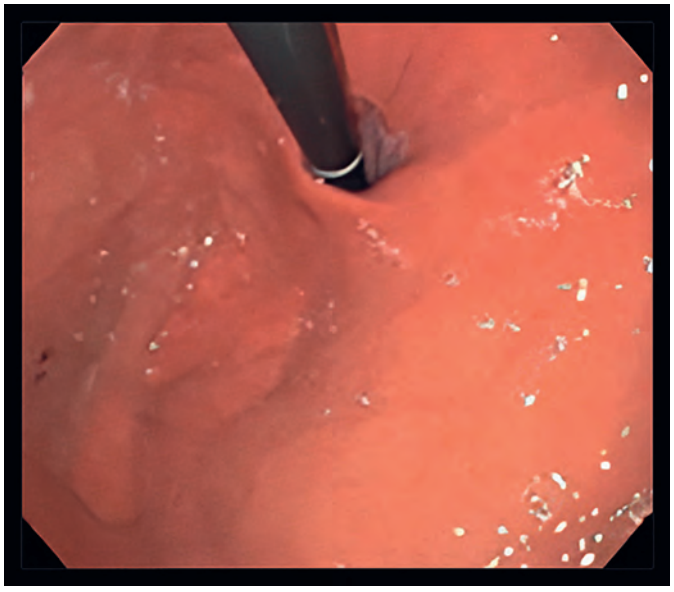


Fig. 1. Endoscopy in retroflexion before anti-reflux mucosal ablation (ARMA).

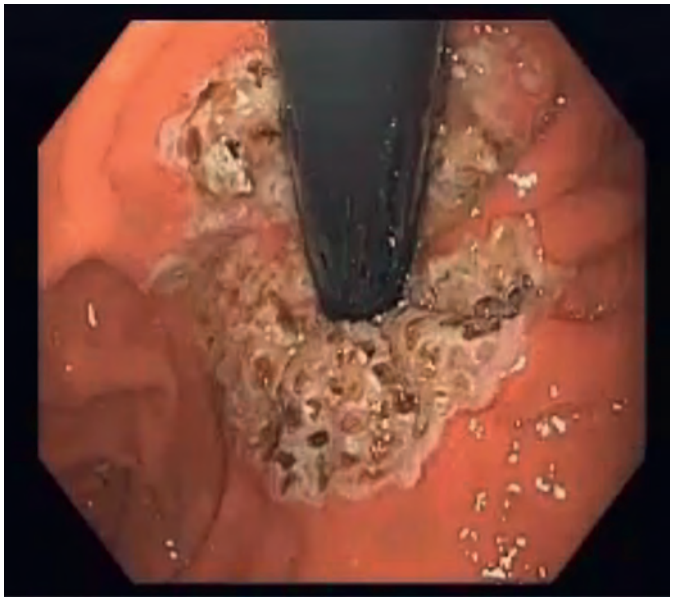


Fig. 2. Endoscopy in retroflexion showing butterfly-shaped mucosal ablation.

sedation, and no complications were noted. The patient received post-procedural double-dose PPIs for 2 months, with subsequent weaning of the medication.

Three months later, he reported significant clinical improvement. The GERD-HRQL score was 21, and the FSSG score was 21. In addition, 24-h pH-impedance test showed normalization of esophageal parameters (acid exposure time: 0.9%, number of reflux episodes: 34, DeMeester score: 3.6). He underwent follow-up esophagogastroduodenoscopy, which showed resolution of the reflux esophagitis, with shrinking effect of the ablated area and grade I Hill's flap valve (Fig. 3). Currently, 2 years post-procedure, the patient remains asymptomatic without PPIs (GERD-HRQL score of 7 and FSSG score of 5).

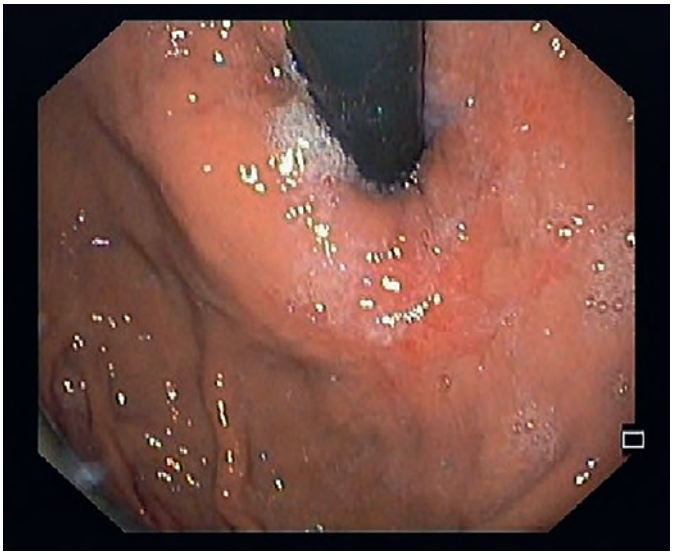


Fig. 3. Endoscopy in retroflexion 3 months after anti-reflux mucosal ablation (ARMA).

GERD is a highly prevalent condition that affects 8–33% of the worldwide population [1]. It can result in several serious complications, including esophageal stricture and Barrett's esophagus, which may increase the risk of esophageal cancer. PPIs have been the cornerstone of the management of these patients. However, 30–40% of patients have persistent GERD symptoms despite PPI therapy [2]. The management of PPIs-refractory GERD has primarily included surgical laparoscopic fundoplication, after excluding other potential causes [3]. However, it is an invasive procedure with potential adverse events. Therefore, less invasive anti-reflux interventions are desired.

Transoral incisionless fundoplication (TIF) is a minimally invasive endoscopic option, which mirrors the Nissen fundoplication by using the EsophyX® device. TIF does not preclude future anti-reflux surgery and can be revised if required. However, it requires proprietary equipment and is a two-operator technique. Long-term outcomes of TIF 2.0 have been showing long-term elimination of GERD symptoms with no severe adverse events [4]. Nonablative radiofrequency treatment involves the application of radiofrequency energy to the muscle fibers of the lower esophageal sphincter and the

gastric cardia through the Stretta® system. The procedure is usually safe and well tolerated, without compromising the possibility of future anti-reflux surgery. Nevertheless, outcome data are heterogeneous, displaying variable response rates [5]. Furthermore, it has been postulated that submucosal injection of inert substances into the gastroesophageal junction may cause tissue remodeling, resulting in improvement of GERD symptoms [6]. Several injectable agents have been evaluated; however, many are no longer available due to poor long-term efficacy and safety concerns. GERDx™ is a new endoscopic device that allows for full-thickness plication [7]. This two-operator technique has a relatively short operating time and a fast learning curve. The experience with the device is still minimal, and the very short follow-up makes this endoscopic treatment experimental at this time. In anti-reflux mucosectomy, endoscopic resection of the gastric cardiac mucosa is performed to reduce the opening of the gastroesophageal junction [8]. It does not require proprietary equipment. However, there is a considerable risk of perforation and bleeding. Given the lack of randomized controlled trials and long-term data, anti-reflux mucosectomy should be reserved for patients included in research protocols.

ARMA is a promising new endoscopic method for PPIs-refractory GERD, patients unwilling or unfit for surgery, after excluding other potential causes of refractory reflux. Recent data have been demonstrating that it is well tolerated and results in gastroesophageal reflux suppression [9]. Moreover, it does not require costly add-on devices and can be performed in a standard endoscopy room. Another important strength of ARMA is that it can be repeated despite presence of fibrosis from previous therapies.

In this case, narrowing the cardia opening using ARMA was a safe and clinical successful procedure, with improved GERD-related symptoms and objective acid reflux parameters. In fact, this technique has been described in the literature as generally effective and safe in patients with refractory GERD, with a significant decrease in the esophageal acid exposure time, number of acid refluxes, and DeMeester score [10]. Nevertheless, randomized comparative studies with long-term follow-up are needed to address the efficacy of this technique.

Statement of Ethics

Written informed consent was obtained from the participant for publication of the details of his medical case and any accompanying images. This type of manuscript (case report) did not require ethical approval due to local laws.

Conflict of Interest Statement

The authors have no disclosures to report.

Funding Sources

The authors have no funding sources to declare.

Author Contributions

Isabel Garrido did literature review and drafted the manuscript. Isabel Garrido, Ana Luísa Santos, Rui Morais, Armando Peixoto, and Guilherme Macedo have critically revised and finalized the manuscript. All authors have approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Bredenoord AJ, Pandolfino JE, Smout AJ. Gastro-oesophageal reflux disease. Lancet. 2013;381(9881):1933–42.
2 Dean BB, Gano AD Jr, Knight K, Ofman JJ, Fass R. Effectiveness of proton pump inhibitors in nonerosive reflux disease. Clin Gastroenterol Hepatol. 2004;2(8):656–64.
3 Yadlapati R, Vaezi MF, Vela MF, Spechler SJ, Shaheen NJ, Richter J, et al. Management options for patients with GERD and persistent symptoms on proton pump inhibitors: recommendations from an expert panel. Am J Gastroenterol. 2018;113(7):980–6.
4 Testoni S, Hassan C, Mazzoleni G, Antonelli G, Fanti L, Passaretti S, et al. Long-term outcomes of transoral incisionless fundoplication for gastro-esophageal reflux disease: systematic-review and meta-analysis. Endosc Int Open. 2021;9(2):E239–46.
5 Aslam N, Telese A, Sehgal V, Sweis R, Lovat LB, Haidry R. Minimally invasive endoscopic therapies for gastro-oesophageal reflux disease. Frontline Gastroenterol. 2023;14(3):249–57.
6 Kushner BS, Awad MM, Mikami DJ, Chand BB, Wai CJ, Murayama KM. Endoscopic treatments for GERD. Ann N Y Acad Sci. 2020;1482(1):121–9.
7 Kalapala R, Karyampudi A, Nabi Z, Darisetty S, Jagtap N, Ramchandani M, et al. Endoscopic full-thickness plication for the treatment of PPI-dependent GERD: results from a randomised, sham controlled trial. Gut. 2022;71(4):686–94.

8 Inoue H, Ito H, Ikeda H, Sato C, Sato H, Phalanusitthepha C, et al. Anti-reflux mucosectomy for gastroesophageal reflux disease in the absence of hiatus hernia: a pilot study. Ann Gastroenterol. 2014;27(4): 346–51.

9 Inoue H, Tanabe M, de Santiago ER, Abad MRA, Shimamura Y, Fujiyoshi Y, et al. Anti-reflux mucosal ablation (ARMA) as a new treatment for gastroesophageal reflux refractory to proton pump inhibitors: a pilot study. Endosc Int Open. 2020;8(2):E133–8.

10 Chou CK, Chen CC, Chen CC, Wu JF, Liao WC, Chiu HM, et al. Positive and negative impact of anti-reflux mucosal intervention on gastroesophageal reflux disease. Surg Endosc. 2023;37(2): 1060–9.

GE – Portuguese Journal of Gastroenterology

Endoscopic Snapshot

GE Port J Gastroenterol 2024;31:364–366
DOI: 10.1159/000535222

Received: September 9, 2023
Accepted: November 1, 2023
Published online: December 12, 2023

Granulation Polyp: A Pitfall for Digital Chromoendoscopy

Luís Correia Gomes[a] Joana Lemos Garcia[a] Sara Mata[b]
Margarida Rajão Saraiva[a] Sandra Faias[a] Isabel Claro[a]

[a]Department of Gastroenterology, Portuguese Institute of Oncology – Lisbon, Lisbon, Portugal; [b]Department Anatomic Pathology, Portuguese Institute of Oncology – Lisbon, Lisbon, Portugal

Keywords
Granulation polyp · Virtual chromoendoscopy · Colonoscopy

Pólipo de granulação: uma falha da cromoendoscopia virtual

Palavras Chave
Pólipo granular · Cromoendoscopia virtual · Colonoscopia

A 71-year-old woman was referred for treatment of a sessile polyp (0-Is) in the sigmoid described as having altered crypt morphology and disrupted vascular pattern on virtual chromoendoscopy, indicative of malignancy. The endoscopic biopsies revealed no dysplasia.

We repeated colonoscopy, and a sessile polypoid lesion (0-Is) adjacent to a diverticulum with a large ostium was identified. The polyp had a pale color and a small ulceration on white-light endoscopy, measuring 12 mm (Fig. 1). Narrow Band Imaging (NBI) assessment revealed the absence of pit pattern and an aberrant vascular pattern with dilated, irregular, tortuous vessels (Fig. 2). Histological assessment (several biopsies) of the lesion revealed polypoid granulation tissue with fibrinoid necrosis, suggestive of ulceration (Fig. 3). The diagnosis of granulation polypoid tissue arising from a colonic diverticulum was established, and due to its benign nature, we decided not to remove it.

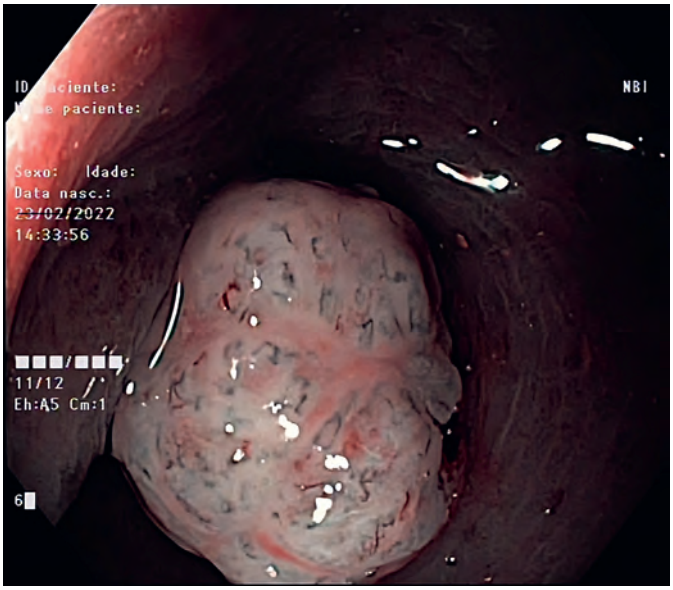


Fig. 1. Sessile polyp (0-Is) with approximately 12 mm located in the sigmoid. It has a pale color and aberrant vessels.

Granulation polypoid tissue is a rare entity with, to our knowledge, just a few case reports on the literature [1–3]. Granulation polypoid tissue could arise from a colon

karger@karger.com
www.karger.com/pjg

Correspondence to:
Luís Correia Gomes, luisfilipe.gomes@outlook.com

diverticulum after recurrent diverticulitis and has no malignant potential, contrary to neoplasms.

These polyps are composed by inflamed granulation tissue and covered by regenerative epithelium and not by colonic epithelium, so there are no crypts on the surface [3]. Subsequently, on virtual chromoendoscopy, they have a fibrotic appearance without pit structures, which could be misinterpreted as the amorphous appearance that characterizes invasive neoplasia [1–4]. In order to differentiate these entities, one should search for other features of neoplasms, despite being nonspecific. These include the presence of an adenomatous component at the periphery or extensive ulceration and friability [4]. Although further investigation is required, according to the similarity of endoscopic images described in the available literature [1–4], we thus speculate that a colonic polyp showing a smooth surface, lack of pit structure, a fibrotic appearance, and aberrant neo-vessels may be typical of a colonic granulation polyp.

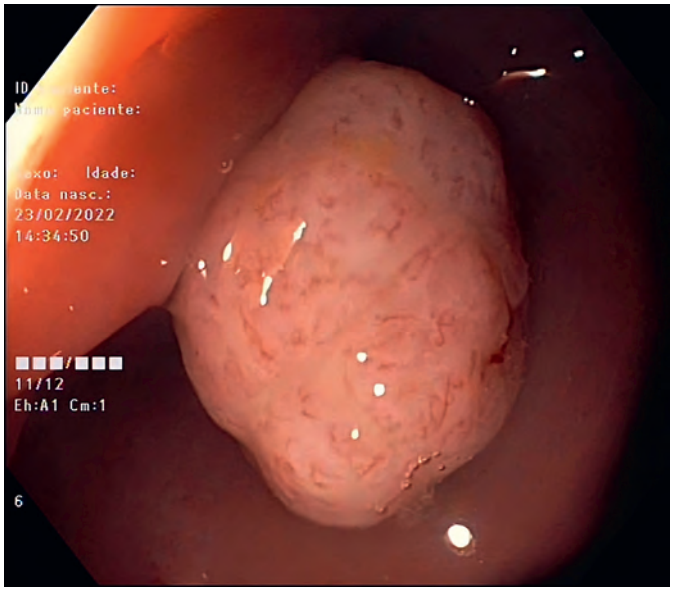


Fig. 2. Narrow band image (NBI) evaluation of the 12 mm sessile polyp, revealing the absence of glandular pattern with aberrant vessels, without evidence of adenomatous tissue.

Statement of Ethics

Ethical approval was not required for this study in accordance with local/national guidelines. The patient gave her written informed consent to publish this case and images.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

None.

Author Contributions

Luís Correia Gomes, Joana Lemos Garcia, and Sandra Faias were involved in the endoscopic procedure and manuscript drafting. Margarida Rajão Saraiva and Sara Mata were involved in manuscript drafting and critical revision. Isabel Claro reviewed the manuscript and gave final approval. All authors approved the final version.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

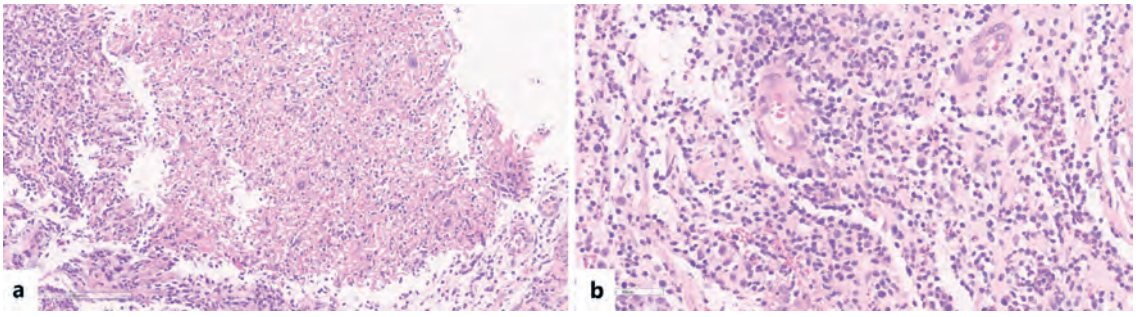


Fig. 3. a Histological findings of biopsies of the polyp. A 10 magnified image with a hematoxylin and eosin (HE) stain, where the blue line reveals fibrinoid necrosis, typical of ulceration. No atypical cells or structural atypia. **b** Histological findings of biopsies of the polyp. A 20 magnified image with a hematoxylin and eosin (HE) stain, which reveals increased outgrowth of microvascular structures and infiltration of lymphocytes, neutrophils, and plasma cells, which indicates granulation tissue. No atypical cells or structural atypia.

References

1 Mori H, Tsushimi T, Kobara H, Nishiyama N, Fujihara S, Matsunaga T, et al. Endoscopic management of a rare granulation polyp in a colonic diverticulum. World J Gastroenterol. 2013; 19(48):9481–4.

2 Zimmer V, Eltze E. Granulation polyps complicating subacute diverticulitis. Gastroenterol Hepatol. 2022 Jun–Jul;45(6): 466–7.

3 Iwamuro M, Takahara M, Yamazaki T, Tanaka T, Kondo Y, Hiraoka S, et al. A granulation polyp in the colon masquerading as metastatic cancer. Acta Med Okayama. 2019; 73(5):457–61.

4 Tanaka S, Sano Y. Aim to unify the narrow band imaging (NBI) magnifying classification for colorectal tumors: current status in Japan from a summary of the consensus symposium in the 79th Annual Meeting of the Japan Gastroenterological Endoscopy Society. Dig Endosc. 2011;23(Suppl 1):131–9.

GE – Portuguese Journal of Gastroenterology

Endoscopic Snapshot

GE Port J Gastroenterol 2024;31:367–369
DOI: 10.1159/000535226

Received: September 22, 2023
Accepted: November 2, 2023
Published online: December 12, 2023

Appendiceal Submucosal Tumor: The Potential of Endoscopic Full-Thickness Resection in a Rare Entity

Catarina Costa[a] Pedro Mesquita[a] Manuela Estevinho[a, b] João Correia[a] Jaime Rodrigues[a] Teresa Freitas[a]

[a]Gastroenterology Department, Vila Nova de Gaia/Espinho Hospital Center, Vila Nova de Gaia, Portugal; [b]Biomedicine Department, Unit of Pharmacology and Therapeutics, Faculty of Medicine, University of Porto, Porto, Portugal

Keywords
Hamartoma · Appendix · Endoscopy · Full-thickness

Lesão subepitelial do apêndice: o potencial da resseção endoscópica transmural numa entidade rara

Palavras Chave
Hamartoma · Apêndice · Endoscopia · Resseção transmural

A 71-year-old man with a previous appendicectomy underwent colonoscopy, after a positive fecal occult blood test (by the immunochemical method), that showed a submucosal lesion in the appendiceal stump. He was referred to our department where an abdominal CT scan was requested with no relevant findings identified. A revaluation colonoscopy was scheduled, where a 10-mm polypoid lesion underneath normal-appearing mucosa, consistent with a submucosal lesion, was identified in the center of the appendiceal orifice (shown in Fig. 1). Standard polypectomy or endoscopic mucosal resection was considered not feasible. We proceeded to endoscopic full-thickness resection (EFTR), using the full-thickness resection device

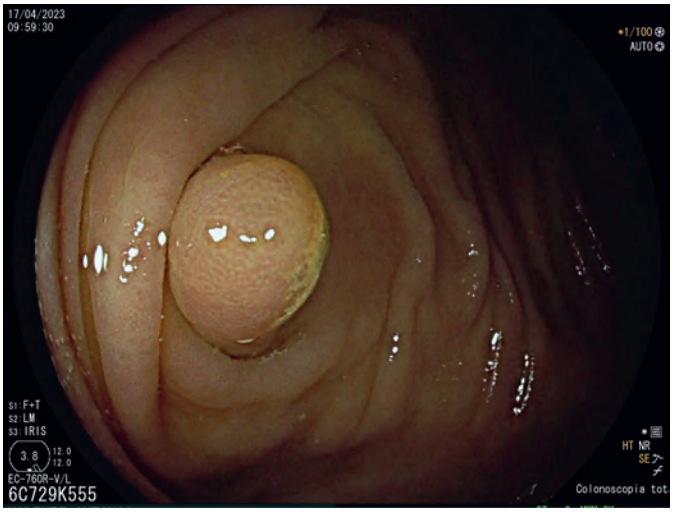


Fig. 1. Colonoscopy image showing a 10-mm submucosal lesion in the center of the appendiceal orifice.

(FTRD, Ovesco®, Germany) (shown in Fig. 2, 3). The patient was discharged 1 h after the procedure, with no symptoms. No prophylactic antibiotics were given. Follow-up was uneventful, without complications. Histopathologic analysis of the lesion revealed a submucosal proliferation of smooth-muscle bundles,

karger@karger.com
www.karger.com/pjg

Correspondence to:
Catarina Costa, catarina.icosta97@gmail.com

mature adipose tissue, and thick-walled tortuous vessels, consistent with a diagnosis of a hamartoma with a R0 resection.

Hamartomatous polyps of the gastrointestinal tract are a rare entity and may be solitary or multiple, the latter often associated with genetic predisposition, such as Peutz-Jeghers syndrome (PJS) and juvenile polyposis. It is vital to distinguish these two identities since the last one involves an increased risk of cancer. Two types of solitary polyps can be identified: Peutz-Jeghers-type solitary polyps that usually appear during the 4th decade of life in patients without family history of PJS and extra-digestive manifestations, such as mucocutaneous hyperpigmentation; and juvenile sporadic polyps, more common in children and mostly located in the rectosigmoid region. Histological diagnosis of hamartomatous polyps is relatively straightforward, but the distinction between particular types may be tricky [1]. In the clinical case here reported, considering the age of the patient, the location of the lesion, and the histology report, a solitary Peutz-Jeghers-type lesion is most likely. There was no familiar history or extra-digestive manifestations that resembled PJS.

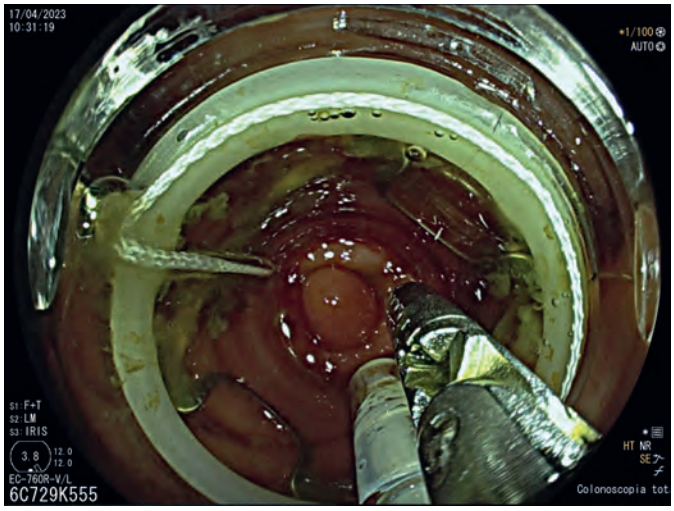


Fig. 2. Device-assisted EFTR procedure (FTRD, Ovesco®, Germany).

Solitary hamartomatous polyps are mostly found in the colon and rarely in the appendix [2], with only a few cases described. They can be asymptomatic and found accidentally or manifest as a complication such as intussusception, which entails a surgical approach [3, 4]. Classically, the primary method of resection of colorectal submucosal lesions was surgery, and an endoscopic approach was not possible for lesions inserted at the inner part of the appendix. Recently, new endoscopic procedures were developed and are gaining more and more acceptance, such as EFTR. This device-assisted technique involves transmural resection of the digestive wall preceded by preemptive clip closure of the future defect. It provides a less invasive approach for management of lesions in the deeper layers of the digestive wall or positioned at complex anatomical sites, such as the appendix, while still achieving clear resection margins [5].

In conclusion, this case highlights a rare identity and the role of EFTR in the management of lesions previously not amenable to endoscopic resection, such as submucosal and/or appendicular lesions. Currently, it should already be regarded as an alternative to surgery in selected patients.

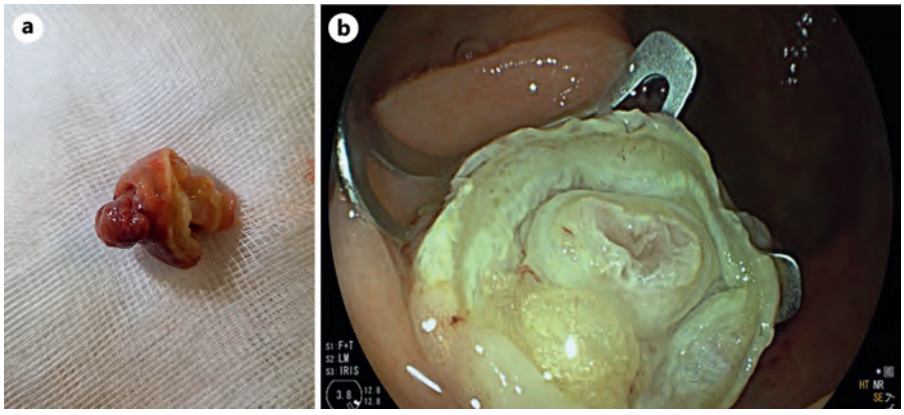


Fig. 3. a Macroscopic image of the resected specimen, with the submucosal lesion on the cecal side, and the appendiceal stump on the opposite side. **b** Endoscopic aspect of the resection site, with the EFTR over-the-scope clip in situ.

Statement of Ethics

Ethics approval was not required. Written informed consent was obtained from the patient for publication of this case report and any accompanying images. All personal details were anonymized.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was not supported by any sponsor or funder.

Author Contributions

Catarina Costa, MD, Pedro Mesquita, MD, Manuela Estevinho, MD, João Correia, MD, and Jaime Rodrigues, MD: participated in the endoscopic procedure, writing – original draft, and writing – review and editing; Teresa Freitas, MD, and head director of the gastroenterology department of our hospital: participated in writing – review and editing.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Cauchin E, Touchefeu Y, Matysiak-Budnik T. Hamartomatous tumors in the gastrointestinal tract. Gastrointest Tumors. 2015;2:65–74.
2 Iwamuro M, Aoyama Y, Suzuki S, Kobayashi S, Toyokawa T, Moritou Y, et al. Long-term outcome in patients with a solitary Peutz-jeghers polyp. Gastroenterol Res Pract. 2019; 2019:8159072–5.
3 Sant'Anna M, Gravito-Soares E, Gravito-Soares M, Mendes S, Figueiredo PN. Solitary peutz-jeghers type hamartomatous polyp arising from the appendix. GE Port J Gastroenterol. 2023;30(2):156–8.
4 Choi CI, Kim DH, Jeon TY, Kim DH, Shin NR, Park DY. Solitary Peutz-Jeghers-type appendiceal hamartomatous polyp growing into the terminal ileum. World J Gastroenterol. 2014; 20(16):4822–6.
5 Dalal I, Andalib I. Advances in endoscopic resection: a review of endoscopic submucosal dissection (ESD), endoscopic full thickness resection (EFTR) and submucosal tunneling endoscopic resection (STER). Transl Gastroenterol Hepatol. 2022;7:19.

GE – Portuguese Journal of Gastroenterology

Clinical Case Study

GE Port J Gastroenterol 2024;31:370–375
DOI: 10.1159/000535814

Received: March 25, 2023
Accepted: October 30, 2023
Published online: January 8, 2024

Endoscopic Management of Dysfunctioning Gastric Band after Sleeve Gastrectomy with the Luso-Cor® Esophageal Stent

Filipe de Sousa Damião[a] Patrícia Santos[a] João Lopes[a] João Raposo[b] Carlos Noronha Ferreira[a, c] Rui Marinho[a, c]

[a]Serviço de Gastrenterologia e Hepatologia, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal; [b]Serviço de Cirurgia, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal; [c]Clínica Universitária de Gastrenterologia, Faculdade de Medicina de Lisboa, Universidade de Lisboa, Lisboa, Portugal

Keywords
Banded sleeve gastrectomy · Gastric band migration · Bariatric surgery endoscopy · Gastric outlet obstruction · Endoscopic stenting

Abstract
Sleeve gastrectomy (SG) can be aided by the addition of a calibration silicone ring, banded SG (BSG). It provides better weight loss than non-banded SG but with higher rate of adverse events. The aim of this case report is to further contribute to the knowledge of how to endoscopically manage these patients by placing a new esophageal stent (Luso-Cor®). A 58-year-old female with grade III obesity (weight 110 kg, BMI: 45.2 kg/m^2) underwent SG in 2013. Due to the limited weight loss, a surgical calibration silicon ring was placed in 2017. In the following months, she developed recurrent and abundant postprandial regurgitation, achieving a minimum weight of 66 kg (BMI: 27.1 kg/m^2). Gastroesophageal transit showed a stricture at the junction of the gastric corpus and antrum, causing gastric outlet obstruction. Endoscopy identified a regular luminal stenosis with normal mucosa, which allowed easy passage of the endoscope with slight pressure. Two sessions of endoscopic dilatation were performed, first with an 18-mm through-the-scope balloon and later with a 30-mm pneumatic balloon without symptomatic relief. A two-step endoscopic therapeutic approach was proposed to first promote intragastric ring erosion by placing a new partially covered metallic stent, Luso-Cor® esophageal stent 30/20/30 × 240 mm, and subsequently retrieve the stent, followed by cutting and retrieval of the ring. The proximal flare with a 30 mm diameter was placed in the distal esophagus and the distal edge in the prepyloric antrum. However, 2 weeks later, she complained of vomiting and abdominal fullness. Complete migration of the proximal flare of the stent into the remnant gastric fundus was seen on the contrast study. Endoscopy was performed, and the stent was easily removed. A blue calibration ring, partially eroded into the gastric lumen, was observed at the site of gastric tube stenosis. After stent removal, the patient was asymptomatic, and so conservative follow-up was decided. A follow-up endoscopy, performed 5 months later, showed complete reepithelization of the eroded ring. The patient remains asymptomatic after 3 years of follow-up and has regained weight up to 76 kg (BMI: 31.2 kg/m^2). The efficacy of endoscopy on the management of ring-related adverse events has been previously reported. Small-case series describe the use of multiple pneumatic dilations or the deployment of plastic or covered metallic stents to cause erosion of the overlying mucosa, followed by cutting and retrieval of the ring. In conclusion, we

karger@karger.com
www.karger.com/pjg

Correspondence to:
Filipe de Sousa Damião, filipe.desousa.damiao@gmail.com

believe that the mural pressure exerted by the Luso-Cor® esophageal stent, in the limited period it remained in situ, was sufficient to relieve the luminal pressure of the silicon ring, realigning the ring with the remnant gastric tube. This rare clinical entity highlights the potential role of specific metallic stents in the management of these patients.

Resolução endoscópica de estenose pós gastrectomia vertical com anel de silicone – utilização de prótese esofágica Luso-Cor®

Palavras Chave
Sleeve gástrico com anel de silastic · Migração de banda gástrica · Endoscopia na cirurgia bariátrica · Obstrução do esvaziamento gástrico · Próteses endoscópicas

Resumo
A cirurgia bariátrica de gastrectomia vertical (*sleeve* gástrico) pode ser complementada pela adição de um anel restritivo de silicone – *sleeve* gástrico com anel de silastic. O acréscimo deste anel promove uma maior perda de peso, no entanto está associado a maior risco de eventos adversos. O objetivo da apresentação deste caso é contribuir para as diferentes técnicas úteis no tratamento das complicações relacionadas com o anel, através da utilização de uma prótese esofágica (Luso-Cor®). Uma doente de 58 anos, com obesidade grau III (peso 110 kg, IMC 45,2 kg/m^2), foi submetida a um *sleeve* gástrico em 2013. Não apresentou perda de peso favorável e, em 2017, foi colocado um anel de silicone rodeando o tubo gástrico. Nos meses seguintes desenvolveu regurgitação pós-prandial recorrente e abundante, alcançando um peso mínimo de 66 kg (IMC 27,1 kg/m^2). Realizou um trânsito gastroesofágico que revelou uma estenose na junção do corpo com o antro gástrico, com evidência de obstrução do esvaziamento gástrico. A endoscopia digestiva alta identificou uma estenose regular recoberta por mucosa sem lesões, com passagem do aparelho após pressão ligeira. Foram realizadas duas sessões de dilatação, inicialmente com balão *trough-the-scope* de 18 mm e posteriormente com balão pneumático de 30 mm. Os sintomas persistiram e, por esse motivo, foi decidido uma abordagem em dois tempos: primeiro promover a erosão intragástrica da banda para depois a seccionar e remover intraluminalmente. Nesse sentido, foi colocada uma prótese metálica esofágica parcialmente coberta, Luso-Cor® 30/20/30 × 240 mm. O segmento proximal da prótese com 30 mm de diâmetro foi colocado no esófago e o bordo distal da prótese ficou no antro pré-pilórico. No entanto, duas semanas depois, a doente queixou-se de vómitos e enfartamento precoce. O estudo radiográfico com contraste revelou migração distal da prótese, com deslocamento do segmento proximal para o corpo gástrico remanescente. A prótese foi removida endoscopicamente sem dificuldade e, na região da estenose, foi observado o anel de silicone parcialmente erosionado para o lúmen gástrico. Após remoção da prótese a doente evoluiu favoravelmente, sem novos sintomas, e, por esse motivo, foi decido seguimento sem novas intervenções. A endoscopia de seguimento, realizada cinco meses após, demonstrou reepitelização completa do anel parcialmente erosionado. A doente permanece assintomática após três anos de seguimento e voltou a ganhar peso (peso atual 76 kg, IMC 31,2 kg/m^2). A eficácia da resolução endoscópica de estenoses relacionadas com anel de silicone no *sleeve* gástrico já foi relatada. Pequenas séries de casos utilizaram múltiplas sessões de dilatação com balão pneumático ou colocação de próteses plásticas ou metálicas cobertas para promover erosão intragástrica do anel e sua remoção. Acreditamos que a pressão mural exercida pela prótese Luso-Cor®, no curto tempo em que permaneceu in situ, foi suficiente para aliviar a obstrução, realinhando o seu diâmetro com o restante tubo gástrico. Através do relato desta entidade clínica rara, esperamos contribuir para o conhecimento das próteses metálicas específicas para o manejo destes doentes.

Introduction

Band-assisted procedures have become one of the most common bariatric surgeries performed worldwide [1]. In sleeve gastrectomy (SG), if weight loss does not achieve the necessary metabolic goal, the surgical addition of a silicon ring to the gastric sleeve (banded SG [BSG]) further increases weight loss [2, 3]. However, it is essential to be aware of the specific band-related adverse events in this subgroup of patients, such as ring slippage and gastric outlet stenosis. Different methods for diagnosis and treatment have been reported [4–9]. The aim of the present case report is to further contribute to the knowledge of how to endoscopically manage these patients by the placement of a new esophageal stent (Luso-Cor®).

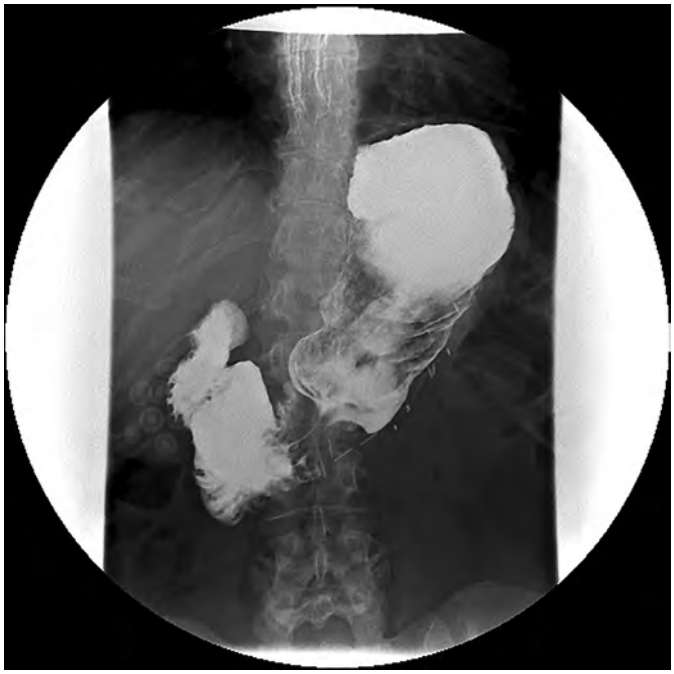


Fig. 1. Gastroesophageal transit with gastric tube stenosis due to silastic ring obstruction.

Case Report

A 58-year-old female with grade III obesity (weight 110 kg, body mass index [BMI] 45.2 kg/m^2) underwent SG in 2013. Due to the limited weight loss, a surgical calibration ring was placed in 2017. In the following months, she developed recurrent and abundant post-prandial regurgitation, achieving a minimum weight of 66 kg (BMI: 27.1 kg/m^2). Gastroesophageal transit showed a stricture at the junction of the gastric corpus and antrum, causing gastric outlet obstruction (Fig. 1). An upper gastrointestinal endoscopy (UGIE) was performed and identified a regular luminal stenosis with normal mucosa, which allowed easy passage of the scope with slight pressure (Fig. 2). Two sessions of endoscopic dilatations were performed, first with an 18-mm through-the-scope balloon and later with a 30-mm pneumatic balloon, without symptomatic relief. A two-step approach was proposed: first to promote intragastric ring erosion by placing a specific partially covered metallic stent, the Luso-Cor esophageal 30/20/30 × 240 mm, and subsequently to cut and endoscopically retrieve the ring. The proximal flare of the stent measuring 30 mm in diameter, which includes a 5-mm uncovered portion, was placed in the distal esophagus and the distal edge in the prepyloric antrum (Fig. 3, 4). However, 2 weeks later, she complained of vomiting and abdominal fullness. Complete migration of the proximal flare into the remnant gastric fundus was seen in the oral contrast study. A new UGIE was performed and the stent was easily removed with a rat tooth forceps. The blue calibration silastic ring, partially eroded into the gastric lumen, was observed at the site of stenosis in the gastric tube (Fig. 5). The patient was discharged and she remained asymptomatic, so we therefore opted for conservative management. The first follow-up UGIE, performed 5 months after the metallic stent removal, showed complete reepithelization of the eroded ring (Fig. 6).

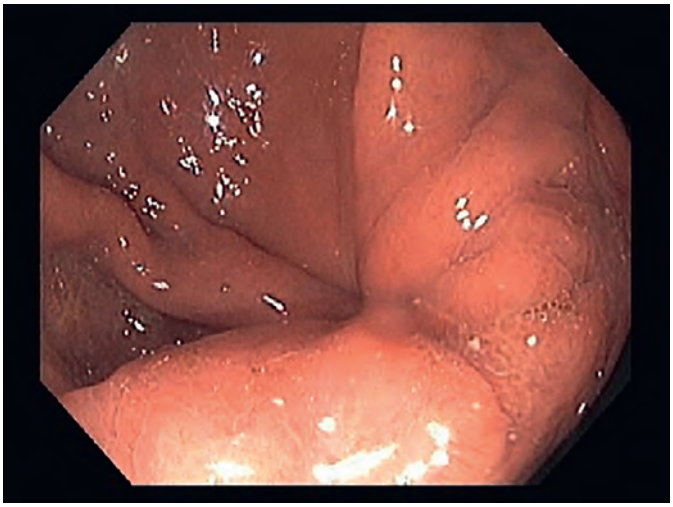


Fig. 2. Endoscopy identifying stenosis in the distal gastric tube with normal overlying mucosa.

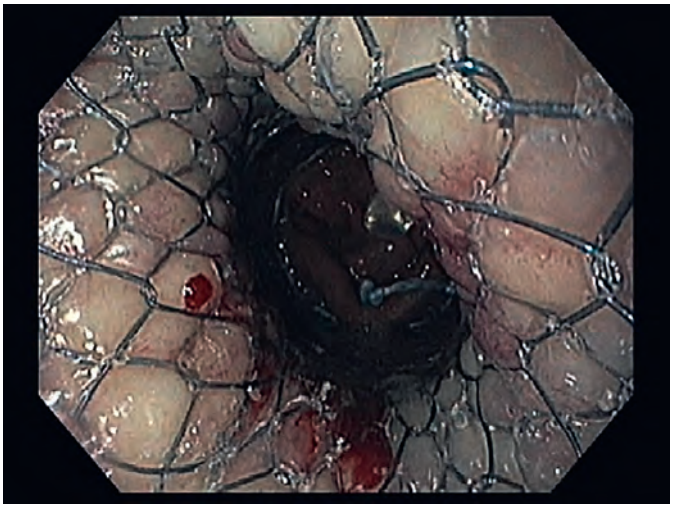


Fig. 3. Endoscopic view of stenosis immediately after stent deployment.

The last UGIE was performed after one and a half years of follow-up and showed gastric stenosis at the site of the ring, without scope passage obstruction. The patient remained asymptomatic after 3 years of follow-up and has regained weight up to 76 kg (BMI: 31.2 kg/m^2).

Discussion

The main bariatric surgical procedures that utilize a silastic ring are banded Roux-en-Y, gastric lap-band, and BSG. Despite well-known metabolic results, several adverse events related to the ring or the band have been

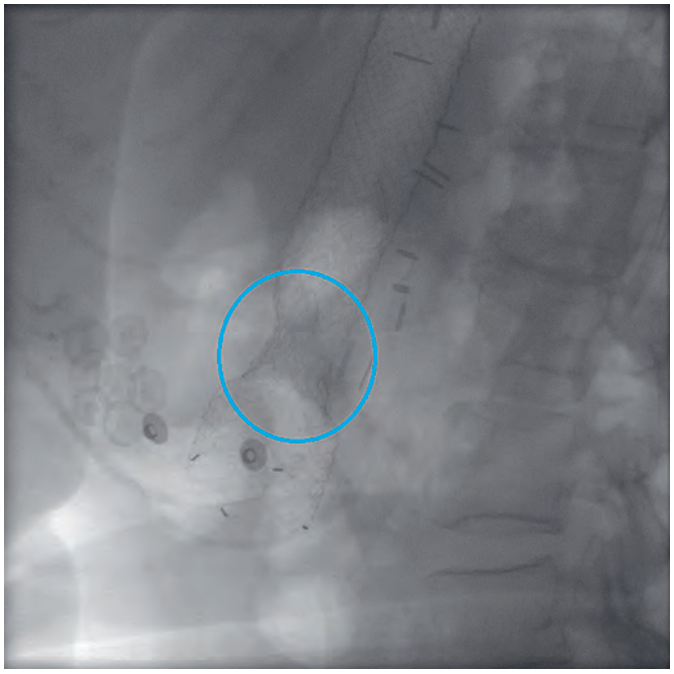


Fig. 4. Fluoroscopic view of stenosis (circle) immediately after stent deployment.

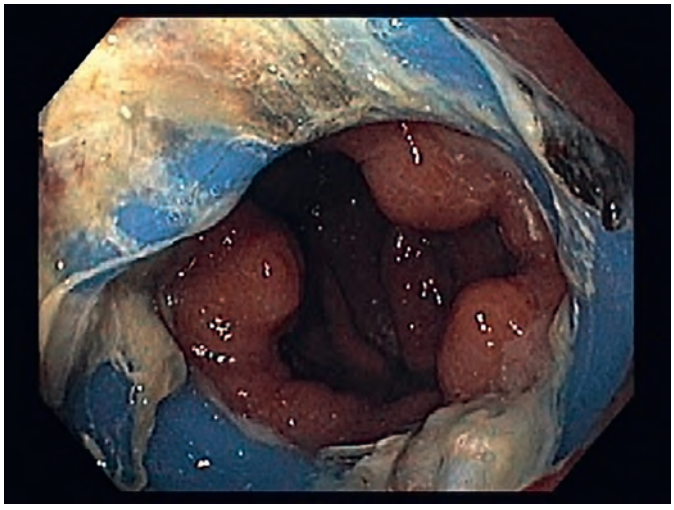


Fig. 5. Erosion of the gastric band due to pressure necrosis of the overlying tissue.

described [4–10]. BSG is associated with a 4% incidence of ring-related adverse events, with stenosis of the gastric tube being the most common [3]. Unlike lap-band, in BSG, the ring is placed loosely around the gastric sleeve to prevent dilation; therefore, it does not permanently compress the gastric wall [3].

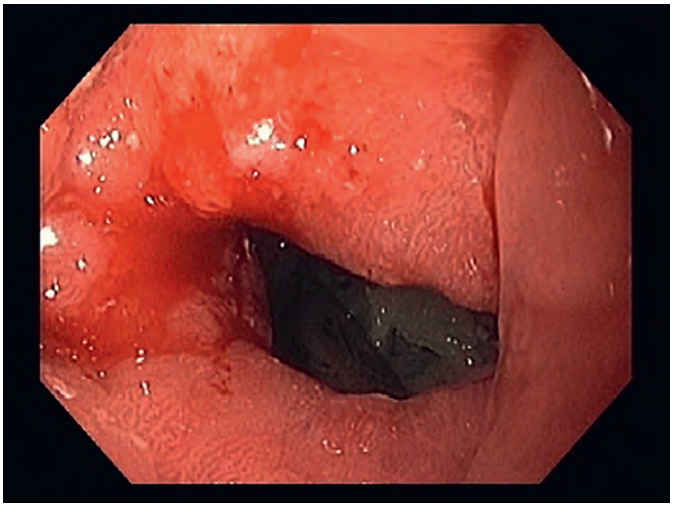


Fig. 6. Complete reepithelization of the eroded ring.

There are mainly four types of band dysfunction. Type I is defined by the slipping of the band, seen in the early stages after surgery. This type of malfunction is not an indication for endoscopic management. Type II ring dysfunction is represented by outlet stoma stenosis of the pouch, and this was what our patient developed. Band erosion of the gastric mucosa is defined as a type III dysfunction: minor erosion represented by type IIIa; major erosion, more than half of the band/ring circumference, represented by type IIIb [4]. Types II, IIIa, and IIIb can be identified later after surgery, and although classically managed by surgery, endoscopic approach has been reported to be safe and feasible [4–6].

Management of band dysfunction by endoscopic dilation has been reported by Campos et al. [6]. They published a series of 35 patients with gastric pouch outlet stenosis (type II dysfunction) due to ring slippage after gastric bypass. All patients were treated with multiple 30 mm pneumatic balloon dilations with a 100% efficacy rate. On average, patients required two sessions of endoscopic dilation. Adverse events occurred in 14.3% (n = 5) and included asymptomatic ring erosion in 11.4% (n = 4) and self-limited upper digestive tract hemorrhage in 2.8% (n = 1). However, all the patients managed in this series had undergone gastric bypass surgery, while our patient underwent BSG. We attempted to relieve the mural ring compression with endoscopic dilatations with balloons with progressively bigger diameter without success.

Stent-induced intragastric band/ring erosion has been previously described with different types of stents [4, 7–9]. In all of the series, a two-step approach was

advocated: with initial deployment of a metallic or plastic stent to progressively induce necrosis and intragastric erosion of the band and a subsequent endoscopy to cut and retrieve the eroded ring [4, 7–9].

One of the few published manuscripts related to metallic stents describes a series of 15 patients. Stents measuring 120–155 mm in length (fully and partially covered) were used. In the first half of the patients, two metal stents were placed, both 120 mm in length and 22–23 mm wide, with a goal of achieving at least 50 mm overlap, to increase circumferential pressure and avoid migration. On the second half, in an effort to avoid esophageal overlap, which seemed to cause pain in some patients, only a 120-mm stent was used but with phalanges to avoid migration. Reported success rates were 87% but with a relatively high adverse event rate of 33%, mainly substernal chest pain, migration, nausea and vomiting, and stricture [7].

Blero et al. [4] described a plastic stent-based approach, specifically in patients after BSG. The study involved a limited number of patients with type II dysfunction (6 patients), but who were all successfully managed with this strategy, without major adverse events [4]. Marins Campos et al. [9] in another study reported the use of the same plastic stents in 41 patients with noneroded rings but in the context of banded Roux-en-Y gastric bypass. The stent remained in place for a mean of 15 days, and the results showed successful ring removal in all patients, no migration, vomiting, or abdominal pain in 22% of patients ($n = 9$), and fibrotic strictures after stent removal in 22% ($n = 9$) treated with endoscopic dilation [9].

Due to the lack of improvement after the initial dilations, unavailability of the plastic stents, and high adverse events of previously published metallic stent methods, we attempted to induce intragastric ring migration with the partially covered Luso-Cor® esophageal metal stent, measuring 240 mm in total length with 30 mm diameter proximal and distal flares and 20 mm main body diameter. We believed that the proximal flare, which includes a 5-mm uncovered portion near the proximal edge, would maintain the stent in the distal esophagus. However, early distal migration of the stent occurred and only partial erosion of the silastic ring was achieved. We hypothesized that if the Luso-Cor® stent had remained for more time, circumferential erosion of the mucosa over the stent could have been possible, since most successful cases report a time of 2–4 weeks for stent placement [4, 7–9]. However, the mural pressure exerted by the Luso-Cor® stent may have been sufficient to relieve the luminal pressure of the silastic ring, realigning the ring with the remnant gastric tube, consequently contributing to the resolution of the patient's symptoms.

In conclusion, band and ring dysfunction after bariatric surgery is a well-known adverse event and the approach to these patients should be multidisciplinary discussed. Endoscopic management is effective but the ideal choice of endoscopic procedure is unclear, with dilation and stents being the most effective. We hope this case report of this rare clinical entity may help to better understand the efficacy of specific covered metallic stents in the management of these patients.

Statement of Ethics

As this was a retrospective analysis of a case report with concealment of the patient's identity, we did not consider it necessary to obtain the approval of the Ethics Committee. Similarly, Ethics Committee approval was not required due to national laws. The patient gave informed consent to perform the endoscopic procedures and the publication of this case report.

Conflict of Interest Statement

The authors have no conflicts of interest to declare. Moreover, they are aware that the manuscript's copyright belongs to *GE – Portuguese Journal of Gastroenterology*.

Funding Sources

No funding resources were used in the elaboration of the article.

Author Contributions

Filipe de Sousa Damião: conception and design, analysis and interpretation of the data, and drafting of the article; Patrícia Santos and Carlos Noronha Ferreira: endoscopic interventions, conception and design, critical revision of the article, and final approval of the article; João Lopes: endoscopic interventions, critical revision of the article, and final approval of the article; João Raposo and Rui Tato Marinho: critical revision of the article and final approval of the article.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Buchwald H, Oien DM. Metabolic/bariatric surgery worldwide 2011. Obes Surg. 2013; 23(4):427–36.
2 Fink JM, Hetzenecker A, Seifert G, Runkel M, Laessle C, Fichtner-Feigl S, et al. Banded versus nonbanded sleeve gastrectomy: a randomized controlled trial with 3 years of follow-up. Ann Surg. 2020;272(5):690–5.
3 Lemmens L, Van Den Bossche J, Zaveri H, Surve A. Banded sleeve gastrectomy: better long-term results? A long-term cohort study until 5 years follow-up in obese and superobese patients. Obes Surg. 2018;28(9):2687–95.
4 Blero D, Eisendrath P, Vandermeeren A, Closset J, Mehdi A, Le Moine O, et al. Endoscopic removal of dysfunctioning bands or rings after restrictive bariatric procedures. Gastrointest Endosc. 2010;71(3):468–74.
5 Collado-Pacheco D, Rábago-Torre LR, Arias-Rivera M, Ortega-Carbonel A, Olivares-Valles A, Alonso-Prada A, et al. Endoscopic extraction of adjustable gastric bands after intragastric migration as a complication of bariatric surgery: technique and advice. Endosc Int Open. 2016;4(6): E673–7.
6 Campos JM, Evangelista LF, Ferraz AA, Galvao Neto MP, De Moura EGH, Sakai P, et al. Treatment of ring slippage after gastric bypass: long-term results after endoscopic dilation with an achalasia balloon (with videos). Gastrointest Endosc. 2010; 72(1):44–9.
7 Wilson TD, Miller N, Brown N, Snyder BE, Wilson EB. Stent induced gastric wall erosion and endoscopic retrieval of nonadjustable gastric band: a new technique. Surg Endosc. 2013;27(5):1617–21.
8 Talib A, de Ridder R, Straathof JW, Bouvy ND. Stent-induced compression necrosis for the endoscopic removal of a partially eroded Lap-Band. BMJ Case Rep. 2018; 2018:bcr2018224670.
9 Marins Campos J, Moon RC, Magalhães Neto GEJ, Teixeira AF, Jawad MA, Bezerra Silva L, et al. Endoscopic treatment of food intolerance after a banded gastric bypass: inducing band erosion for removal using a plastic stent. Endoscopy. 2016;48(6):516–20.
10 Buchwald H, Buchwald JN, McGlennon TW. Systematic review and meta-analysis of medium-term outcomes after banded roux-en-Y gastric bypass. Obes Surg. 2014;24(9): 1536–51.

Explore our products and services

FOR RESEARCHERS

Supporting the Scientific Community at Each Step

IA23008_280

Karger