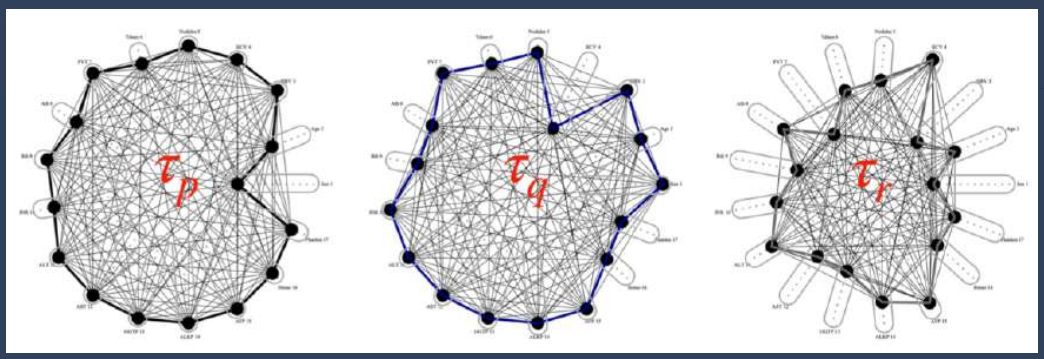
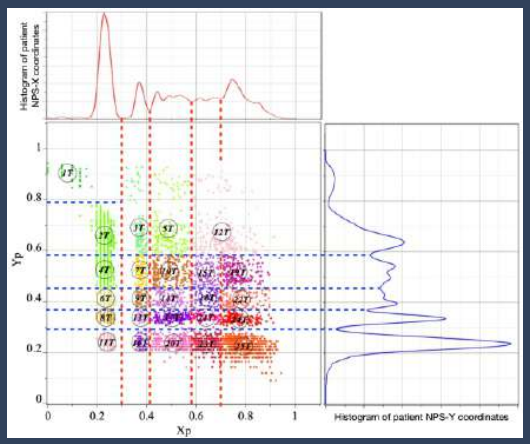
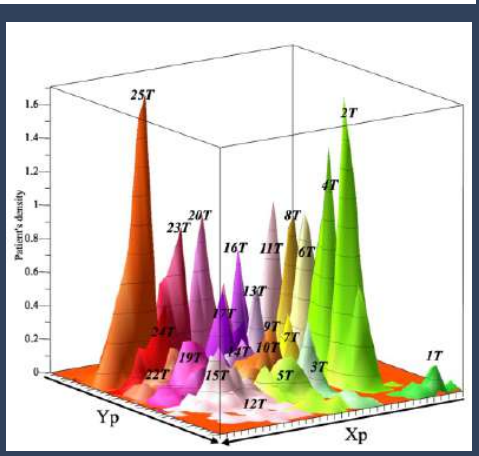
The Official Journal of the Inonu Liver Transplantation Institute

Journal of Inonu Liver Transplantation Institute







A New Approach to Analysis of Clinical Data and Prognostication for Patients with Hepatocellular Carcinoma, based upon a Network Phenotyping Strategy (NPS) Computational Method

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Journal of Inonu Liver Transplantation Institute

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Aim and Scope: The Journal of Inonu Liver Transplantation Institute is a peer-reviewed open-access e-only publication in the field of liver transplantation publishing research articles on clinical, experimental liver transplantation, combined liver and other organ transplantation, and liver diseases. The journal welcomes original research articles, reviews, meta-analyses, case reports, and letters.
Average Duration of the First Review Round: 2 months
Type of Publications: Research Article, Review Article, Meta-Analyses, Case Report, Letter to the Editor
Language of Publication: English
Frequency: 3 issues per year
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JOURNAL OF INONU LIVER TRANSPLANTATION INSTITUTE

Aim and Scope

Aim

The Journal of Inonu Liver Transplantation Institute is a peer-reviewed open-access e-only publication in the field of liver transplantation publishing research articles on clinical, experimental liver transplantation, combined liver and other organ transplantation, and liver diseases. The journal welcomes original research articles, reviews, meta-analyses, case reports, and letters.

Overview

Journal of Inonu Liver Transplant Institute has been founded and established by Inonu Liver Transplant Institute in order to form a source of high-quality research in diseases and therapy of the liver and biliary tract. Both clinicians and basic science researchers are the target population of our journal.

Scope

Hepatobiliary disorders are a complex spectrum of diseases, usually requiring a multi-disciplinary approach that involves interventional radiologists, hepatologists, oncologists, hepatobiliary-transplant surgeons and translational researchers. The Journal of Inonu Liver Transplant Institute (JILTI) is internationally peer reviewed and provides a source for articles on prevention, diagnosis and cutting-edge therapy of hepatobiliary diseases and cancers which also includes liver transplantation, complex hepatobiliary surgical procedures, medical and immune therapies. In accordance with our aims, basic and translational research as applied to these diseases have utmost importance for our journal.

Keywords: Hepatobiliary diseases and cancers, liver surgery, liver transplantation, advanced therapy of hepatobiliary diseases, basic and translational research on hepatobiliary diseases.

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Journal of Inonu Liver Transplantation Institute

Ethical Responsibilities and Policies

Fee - Charges

The Journal of Inonu Liver Transplantation Institute (The Journal) assesses NO submission fee, publication fee (article processing charges - APC), or page charges.

Publication Ethics

The Journal applies standards throughout the publication process to further our goal of sharing high-quality, objective, reliable, and useful information. We implement these processes to ensure appropriate support for our authors and their institutions, as well as our readers. It is crucial that all of the stakeholders in the process (authors, readers and researchers, publishers, reviewers, and editors) comply with ethical principles.
The Journal is an open access publication and follows the guidelines and policies published by the Committee on Publication Ethics (COPE) (https://publicationethics.org). We expect all participants to observe the ethical responsibilities presented below.

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- Studies submitted for publication must be original works of the author. References to other studies must be cited and/or quoted completely and accurately;
- Only those who provide a substantial intellectual contribution to the content of the work may be cited as an author. Other contributors may be recognized with acknowledgements at the conclusion of the article;
- Competing interests or relationships that may constitute a conflict of interest must be declared and explained in all studies submitted for publication;
- Authors must be able to provide documentation showing that they have the right to use the data analyzed, the necessary permissions related to the research, and any appropriate consent;
- Raw data used in the article must be available and may be requested from the author(s) within the framework of the evaluation process;
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- Authors may not submit their article for publication to more than one journal simultaneously. Each application must be initiated following the completion of any previous effort. The Journal will not accept previously published articles;
- Changes in authorship designation (such as adding authors, changing the printed order of the authors, removing an author) once the evaluation process has begun will not be accepted in order to protect all parties involved.

Editor's Role and Responsibilities

General Duties

The editor is responsible for everything published in the journal. In the context of this responsibility, editors have the following duties and obligations:

- Endeavor to meet the needs of readers and authors;
- Maintain continuous development to improve the quality of the journal;
- Consistently work to ensure quality;
- Support freedom of thought;
- Ensure academic integrity;
- Prevent business needs from compromising intellectual and ethical standards;
- Demonstrate clarity and transparency with any necessary corrections or explanations.

Reader Relationship

The editor is to make publication decisions based on expectations of suitable and desirable material. Studies accepted for publication must be original contributions that benefit the reader, researcher, practitioner, and the literature. In addition, editors are obliged to take into account feedback from readers, researchers, and practitioners, and to provide an informative response. Readers will also be informed of any funding provided to support published research.

Author Relationship

- The decision to accept an article is to be based on the importance, original value, validity, and clarity of expression of the work, and the goals and objectives of the journal;
- Studies accepted for evaluation and publication will not be withdrawn unless serious problems are identified;
- The editor will not disregard positive reviewer comments unless there is a serious problem with the study;
- New editors will not change publishing decisions made by previous editor(s) unless there is a serious problem;
- A description of the submission and evaluation process is publicly available;
- Authors are provided with descriptive and informative feedback.

Reviewer Relationship

Reviewers are to be selected according to the subject of the study;
Information and guidance for the evaluation phase is provided;
Any conflicts of interest between authors and reviewers will be disclosed and managed appropriately;
Reviewer identity is to be kept confidential to preserve a blind review process;
Reviewers are to evaluate the study using unbiased, scientific, and constructive comments. Unkind or unscientific commentary will not be permitted;
Reviewers will be evaluated using criteria such as timely response and quality of observations;
The pool of reviewers is to be assessed and supplemented regularly to ensure a broad scope of expertise.

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The editor works with the members of the editorial board to ensure that they are familiar with journal policies and developments in regular meetings and announcements, and will provide training for new members and assistance to board members during their tenure in their role as a supporter of the journal.

- Editorial board members must be qualified and able to contribute to the journal;
- Members of the editorial board must evaluate studies impartially and independently;
- Editorial board members with the appropriate expertise will be given the opportunity to evaluate suitable articles;
- The editor will maintain regular contact with the editorial board and hold regular meetings regarding the development of editorial policies and other aspects of journal management.

Relations with the Owner of the Journal and the Publisher

The relationship between the editors and the publisher/journal owner is based on the principle of editorial independence and stipulated by contract.

Editorial and Blind Review Processes

The editor will apply the publicly defined publication policies created and enforced to ensure a timely and impartial evaluation process for all submissions.

Quality Assurance

The editor is responsible for confirming that the The Journal publishing policies and standards are upheld for all articles.

Protection of Personal Data

The editor is obliged to ensure the protection of personal data related to subjects or images included in published work. Explicit documented consent of the individuals referenced in the research is required before the study will be accepted. The editors is also responsible for protecting the individual data of authors, reviewers, and readers.

Ethics Committee, Human and Animal Rights

The editor is required to ensure that human and animal rights were protected in the studies submitted for publication.

Measures Against Potential Misconduct

The editor must take action against any allegations of possible misconduct. In addition to conducting a rigorous and objective investigation of complaints, the editor is expected to share the findings and conclusions.

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The editor is expected to ensure that any errors, inconsistencies, or misleading statements are corrected quickly and appropriately acknowledged.

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The editor is obliged to protect intellectual property and to defend the rights of the journal and author(s). In addition, the editor is to take the necessary measures to prevent any violation of the intellectual property rights of others in journal publications.

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- Constructive criticism is to be encouraged;
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Conflicts of Interest

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Peer review of research embodies the scientific method, subjecting the work to the rigorous scrutiny of knowledgeable colleagues. The rigor of the review process directly affects the quality of the literature; it provides confidence in an objective and independent evaluation of the published work. The Journal uses a double-blind review process. All comments and the evaluation are transmitted through the journal management system. Reviewers should:

- Only agree to evaluate studies related to their specialty;
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- Refuse to review any work with a potential conflict of interest and inform the journal editor;
- Maintain confidentiality of all information. Only the final published version may be used for any purpose;
- Use thoughtful and constructive language. Hostile or derogatory comments are not acceptable;
- Report any potentially unethical behavior or content to kare@karepb.com via e-mail.

JOURNAL OF INONU LIVER TRANSPLANTATION INSTITUTE

Information for the Authors

THE JOURNAL

The Journal of Inonu Liver Transplantation Institute (The Journal) is an international, scientific, open access periodical published in accordance with independent, unbiased, and double-blinded peer-review principles. The journal is the official publication of the Inonu Liver Transplantation Institute, and it is published in April, August and December, three times a year. The publication language of the journal is English.

The Journal aims to contribute to international literature by publishing high-quality manuscripts in the field of diseases and therapy of the liver and biliary tract. The journal's target audience includes academics and expert physicians working in transplantation surgery specialists.

REVIEW PROCESS

Manuscripts submitted to the Journal will undergo a double-blind peer-review process. Each submission will be reviewed by at least two external, independent peer reviewers who are experts in their field in order to ensure an unbiased evaluation process. The editorial board will invite an external and independent editor to manage the evaluation process of manuscripts submitted by editors or by the editorial board members of the journal. The editor-in-chief is the final authority in the decision-making process for all submissions.

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AUTHORSHIP

Each individual listed as an author should fulfill the authorship criteria recommended by the International Committee of Medical Journal Editors (ICMJE - www.icmje.org). The ICMJE recommends that authorship be based on the following 4 criteria:

Substantial contributions to the conception or design of the work, or the acquisition, analysis, or interpretation of data for the work; AND

Drafting the work or revising it critically for important intellectual content; AND

Final approval of the version to be published; AND

Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

In addition to being accountable for their own work, authors should have confidence in the integrity of the contributions of their co-authors and each author should be able to identify which co-authors are responsible for other parts of the work.

All of those designated as authors should meet all four criteria for authorship, and all who meet the four criteria should be identified as authors. Those who do not meet all four criteria should be acknowledged on the title page of the manuscript.

The Journal requires that corresponding authors submit a signed and scanned version of the authorship contribution form (available for download through www.jilti.org) during the initial submission process in order to appropriately indicate and observe authorship rights and to prevent ghost or honorary authorship. If the editorial board suspects a case of "gift authorship," the submission will be rejected without further review. As part of the submission of the manuscript, the corresponding author should also send a short statement declaring that they accept all responsibility for authorship during the submission and review stages of the manuscript.

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The Open Researcher and Contributor ID (ORCID) number of each author must be submitted when creating an account for correspondence. To obtain an ORCID number, please visit https://orcid.org/

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This journal assesses no submission fees, publication fees, or page charges.

MANUSCRIPT PREPARATION

Manuscripts should be prepared in accordance with the ICMJE-Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (updated in December 2015 - http://www.icmje.org/icmje-recommendations.pdf). Authors are required to prepare manuscripts in accordance with the Consolidated Standards of Reporting Trials (CONSORT) guidelines for randomized research studies, the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines for observational original research studies, the Standards for Reporting Diagnostic Accuracy (STARD) guidelines, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines for experimental animal studies, and the Transparent Reporting of Evaluations with Non-randomised Designs (TREND) guidelines for non-randomized behavioral and public health evaluations.

Manuscripts may only be submitted through the journal's online manuscript submission and evaluation system, http://jag.journalagent.com/jilti/ Manuscripts submitted via any other medium will not be evaluated.

Manuscripts will first be submitted to a technical evaluation process in which the editorial staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines. Submissions that do not conform to the journal's guidelines will be returned to the author with requests for technical correction.

The quality and clarity of the language used in a manuscript is very important. The editors may request that authors have the manuscript professionally edited if the language of the submission does not conform to the journal standards. The Journal uses American English. Please submit text of a quality ready for publication. Information about language editing and copyediting services pre- and post-submission may contact Kare Publishing at kare@karepb.com. Please refer to specific formatting requirements noted in the submission checklist and elsewhere in this document.

MANUSCRIPT TYPES

Original Article: This is the most valued type of article, since it provides new information based on original research. The main text of an original article should be structured with Introduction, Methods, Results, Discussion, and Conclusion subheadings. Original articles are limited to 3500 words and 30 references.

Editorial comment: Editorial comments provide a brief critical commentary offered by reviewers with experience and standing in the topic of a research article previously published in the journal. The authors are selected and invited by the journal to provide the benefit of their expertise. The submission should not include an abstract, keywords, tables, figures, and images. The word count is limited to 1200 and 15 references may be included.

Review article: Two kinds of review are accepted for publication in the Journal: narrative review and systematic review. Reviews of relevant topics not recently discussed in this format that will be helpful to readers are welcomed.

Case report: There is limited space for case reports and therefore the journal selects reports of rare cases or conditions that reflect challenges in diagnosis and treatment, those offering new therapies or revealing knowledge not in the literature, or present something otherwise particularly interesting and educative. The abstract with structured of background, case and conclusion, is limited to 150 words and the report must include the subheadings of introduction, case report, and discussion, which includes a conclusion. A case report is limited to 1300 words and 15 references.

Image: Original, high-quality clinical or laboratory images will be considered for publication. If a photo of an identifiable patient is used, a consent form for its use must be completed and signed by the patient and enclosed with the submission. All printed information that might identify the patient or the authors' institution (including, but not limited to the hospital or patient name, date, or place) should be removed from images. The submission should have no more than 3 authors, the case description is limited to a maximum of 200 words, the discussion section may contain no more than 200 words, and only 3 references and 3 figures are permitted.

Letter to the editor: This type of manuscript discusses important observations, overlooked aspects, or details lacking in a previously published article. Noteworthy articles on subjects within the scope of the journal, particularly educative cases, may also be submitted in the form of a "Letter to the editor." No abstract, keywords, tables, figures, images, or other media should be included. The article that is the subject of commentary must be properly cited within the manuscript. The text should be unstructured and is limited to 500 words. No more than 5 references will be accepted.

Table 1. Limitations for each manuscript type.

Type of manuscript	Wordlimit	Abstract word limit	Referencelimit	Table limit	Figure limit
Original Article	3500	350 (Structured)	40	6	6
Review Article	5000	350	50	6	10
Meta analysis	5000	350	50	6	10
Caser Report	1500	200	20	No tables	5
Letter to the Editor	1000	No abstract	10	No tables	1

Title page: A separate title page should be submitted with all submissions and this page should include:

The full title of the manuscript as well as a short title (running head) of no more than 50 characters

Name, affiliation, ORCID ID number, and highest academic degree of the author(s)

Funding and other material support

Name, address, phone number(s), fax number, and email address of the corresponding author

Acknowledgment of the individuals who contributed to the preparation of the manuscript but who do not fulfill the authorship criteria

Manuscripts that have been presented orally or as a poster should include the name, date and place of the event

Abstract: An English-language abstract is required with all submissions except editorial comments, images, and letters to the editor. Systematic reviews and original articles should contain a structured abstract of maximum 250 words with the subheadings of objective, methods, results, and conclusion.

Keywords: Each submission must be accompanied by a minimum of three and a maximum of six keywords for subject indexing included at the end of the abstract. The keywords should be listed in full without abbreviations. The keywords should be selected from the National Library of Medicine, Medical Subject Headings database (https://www.nlm.nih.gov/mesh/MBrowser.html).

Tables: Tables should be uploaded as separate files and not embedded in the main text. They should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the table with footnotes, even if they are defined within the main text. Tables should be created using the "insert table" command of the word processing software and they should be designed for easy reading. Data presented in tables should not be a repetition of the data presented within the main text but should support the main text.

Figures and figure legends: Figures, graphics, and photographs should be submitted as separate files in TIFF or JPEG format through the article submission system. The files should not be embedded in a Word document or the main document. When there are figure subunits, the subunits should not be

merged to form a single image. Each subunit should be submitted separately through the submission system. Images should not be labeled (a, b, c, etc.) to indicate figure subunits. Thick and thin arrows, arrowheads, stars, asterisks, and similar marks can be used on the images to support figure legend. Like the rest of the submission, the figures should be blind. Any information within the images that may identify an individual or institution should be blinded. The minimum resolution of each submitted figure should be 300 DPI. To prevent delays in the evaluation process, all submitted figures should be clear in resolution and large in size (minimum dimensions: 100x100 mm). Figure legends should be listed at the end of the main document.

All acronyms and abbreviations used in the manuscript should be defined at first use, both in the abstract and in the main text. The abbreviation should be provided in parentheses following the definition. Units should be prepared in accordance with the International System of Units (SI). When a drug, device, hardware, or software program, or other product is mentioned within the main text, the name of the product, the manufacturer/copyright holder of the product (not simply the vendor), and city and the country of the company (including the state, if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric Co., Boston, MA, USA)"

All references, tables, and figures should be referred to within the main text, and they should be numbered consecutively in the order they are referred to within the main text.

Limitations, drawbacks, and shortcomings of original articles should be mentioned in the Discussion section before the conclusion paragraph.

References: The editorial team may request that the authors cite related recently published articles (preferably within the last 10 years) in their manuscripts, with the exception of historical papers.

If an ahead-of-print publication is cited, the digital object identifier (DOI) number should be provided. Authors are responsible for the accuracy of references. Journal titles should be abbreviated in accordance with the journal abbreviations in the Index Medicus /MEDLINE/ PubMed. When there are six or fewer authors, all authors should be listed. If there are seven or more authors, the first six should be listed followed by "et al." In the main text of the manuscript, references should be cited using Arabic numerals in parentheses. The reference styles for different types of publications are presented in the following examples.

Journal article: van Erk MD, Dam-Vervloet AJ, de Boer FA, Boomsma MF, van Straaten H, Bosschaart N. How skin anatomy influences transcutaneous bilirubin determinations: an in vitro evaluation. Pediatr Res 2019;86:471-7.

Epub ahead-of-print article: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead-of-print].

Manuscript published in electronic format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlEID/cid.htm.

Book section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

Books with a single author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or technical report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

REVISIONS

When submitting a revised version of a paper (include a clean copy and a highlighted copy), the author must submit a detailed response to the reviewers that replies to each issue raised by the reviewers and indicates where changes can be found (each reviewer's comment, followed by the author's reply and line number where changes have been made) as well as an annotated copy of the main document. Revised manuscripts must be submitted within 30 days from the date of the decision letter. If the revised version of the manuscript is not submitted within the allocated time, the revision option may be withdrawn. If the submitting author(s) believe that additional time is required, they should request this extension within the initial 30-day period.

Accepted manuscripts are copy edited for grammar, punctuation, format, and clarity. Once the publication process of a manuscript is completed, it is published online on the journal's webpage as an ahead-of-print publication before it is included in the scheduled issue. A PDF proof of the manuscript is sent to the corresponding author and their publication approval is requested within 2 days of receipt of the proof.

PUBLICATION PROCESS

Accepted manuscripts will be made available and citable online as rapidly as possible. The stages of publication are as follows;

Uncorrected publication: A PDF of the final, accepted (but unedited and uncorrected) paper will be published online on the journal web page under the "Accepted Articles" section. A DOI will be assigned to the article at this stage.

Ahead-of-print publication: After copy editing, typesetting, and review of the resulting proof, the final corrected version will be added online in the "Ahead-of-Print" section.

Final publication: The final corrected version will appear in an issue of the journal and added to the journal website. To ensure rapid publication, we ask authors to provide your publication approval during the proofreading process as quickly as possible, and return corrections within 48 hours of receiving the proof.

SUBMISSION CHECKLIST

Please use this list and the following explanations to prepare your manuscript and perform a final check before submission to ensure a timely review.

Formatting of text

- Text should be written in 12-point Times New Roman font
- Main headings and subheadings should be in 12-point and bold font
- Type a single space at the end of each sentence
- Do not use bold face for emphasis within the text
- Numbers one to ten are written out in words unless they are used as a unit of measurement, except in figures and tables
- Use a single hard return to separate paragraphs. Do not use tabs or indents to start a paragraph
- Do not use software options for hyphenation, headers, or footers
- Use page numbering
- Use line numbers
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Ensure that the following items are present:

- **Cover letter**
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  - Article type
  - Article title
  - Running title
  - All author names and affiliations
  - One author has been designated as the corresponding author with contact details
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- Acknowledge
- Manuscripts that have been presented orally or as a poster must include the name of the event, the date, and the location
- State financial or other support for the study
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- **Main text of the manuscript must include:**
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  - Keywords
  - Text with required subheadings
  - References (ensure written according to journal rules)
  - Figures and tables
- Numbered according to text citation
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- Ensure all figure and table citations in the text match the files provided
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- **Ensure that the following forms have been properly completed and submitted:**
- ICMJE Potential Conflict of Interest Disclosure Form (completed by all contributing authors), AND
- Copyright Transfer Form, AND
- Author Contributions Form

These forms are available for download at www.jilti.org

- **Further review**
  - Check the statistical analysis
  - Use the US English spell check and grammar check software functions
  - Check that all references cited in the text are correctly listed in the reference list
  - Permission has been obtained for use of copyrighted material from other sources (including the Internet)
  - All abbreviations have been identified
  - All figures and tables are correctly labeled
  - Journal policies detailed in this guide have been followed.

JOURNAL OF INONU LIVER TRANSPLANTATION INSTITUTE

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Review

Non-Alcoholic Fatty Liver Disease in Living Liver Transplantation: Defatting Strategies

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Abstract

Liver transplantation (LT) is the only treatment option saving lives of the patients who have end-stage liver failure. The disparity between the number of patients waiting for transplantation and that of available cadaveric donors is increasingly being bridged through living donors. However, considering the post-transplantation process, graft selection remains a critical determinant of post-transplant outcomes. Despite the high demand and mortality on waiting lists, numerous donors are rejected due to non-alcoholic fatty liver disease (NAFLD). Consequently, defatting strategies are vital and promising for addressing organ shortage. NAFLD might recur following the transplantation or develop de novo in patients who underwent transplantation operation for other liver diseases. The development of de novo or recurrent NAFLD is closely related with metabolic risk factors and use of immunosuppressive medications by recipients. However, no pharmacological treatment specifically approved for NAFLD exists. Therefore, a multidisciplinary approach is necessary both before and after LT. This review aims to evaluate strategies for preventing NAFLD and implementing defatting techniques in living liver transplant donors and recipients.

Keywords: Living liver transplantation, NAFLD, defatting strategy

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Non-alcoholic fatty liver disease (NAFLD) encompasses a wide clinical spectrum, ranging from steatohepatitis, characterized by fat accumulation in over 5% of hepatocytes, independent of excessive alcohol consumption (≥30 g/day for males, ≥20 g/day for females), to liver cirrhosis and hepatocellular carcinoma (HCC).[1,2] NAFLD, the "hepatic epidemic of the 21st century," affects approximately 25% of the global population.[3] Liver cirrhosis and/or HCC secondary to NAFLD is the second most common etiology among liver transplant candidates on the waiting list.[4]

LT is the only treatment option saving the lives of patients who have end-stage liver failure. Successful outcomes after transplantation depend heavily on graft selection. Steatotic grafts are more vulnerable to cold ischemic injury, which results in an increase in the risks of graft dysfunction, loss, and retransplantation.[5] Fat infiltration is considered treatable for living donors. Defatting strategies, aiming to reduce lipid content in steatotic grafts, have the potential to alleviate organ shortages by significantly expanding the donor pool.[6]

NAFLD can recur after LT or develop de novo in recipients who underwent transplantation due to other liver diseases.[7] Post-transplant steatosis is significantly related with metabolic syndrome, diabetes mellitus (DM), hypertension, and dyslipidemia.[7,8] Immunosuppressive drugs exacerbate

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these metabolic risk factors, further contributing to the development of post-transplant steatosis.[9] This review focuses on strategies for preventing NAFLD and exploring defatting interventions in living donor liver transplantation (LDLT).

1. NAFLD

1.1. Histopathology of NAFLD

Steatosis, which is characterized by triacylglycerol accumulation within hepatocytes, is commonly seen in many liver biopsies. Lipids are estimated to constitute up to 5% of liver parenchyma. Thus, levels of lipid content higher than 5% of liver mass can be seen as "pathological".[10] Macrovesicular steatosis is defined as lipid droplet accumulation within hepatocytes, causing peripheral displacement of the cell nucleus (Fig. 1).[11]

In contrast, microvesicular steatosis involves centrally located nuclei within hepatocytes exhibiting a characteristic "foamy" appearance (Fig. 2).[10,12] The etiologies of macrovesicular and microvesicular steatosis are presented in Table 1.[13]

Drug-induced hepatic steatosis is a rare condition caused by the direct toxic impacts of a medication on the liver. It is estimated that approximately 2% of steatosis cases are drug-induced. Medications can lead to both microvesicular and macrovesicular steatosis. Drug-induced hepatic steatosis is closely related to the duration and dosage of medication use. The medications associated with macrovesicular and/or microvesicular steatosis are illustrated in Figure 3.[2,14]

1.2. Clinical Course of NAFLD

NAFLD encompasses a wide clinical spectrum, ranging between simple steatosis, steatohepatitis, liver cirrhosis, and HCC.[1,2] Non-alcoholic steatohepatitis (NASH), observed in 20% of NAFLD patients, is characterized by hepatic steatosis (>5%) with inflammation and ballooning degeneration. NASH is related to a higher risk of fibrosis.[22,13] Approximately 15–25% of NASH cases progress to cirrhosis, which is related with the development of HCC and increased mortality. The progression of NAFLD from a healthy liver to HCC is illustrated in Figure 4.[16]

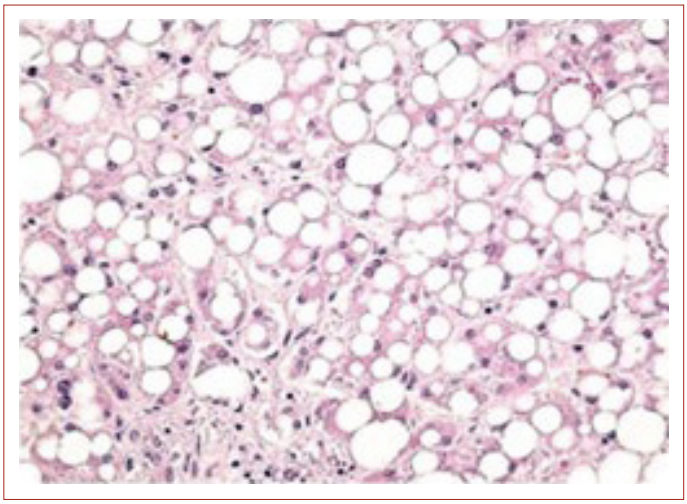

Figure 1. Macrovesicular steatosis.

2. Donor Steatosis in LDLT

2.1. Pre-Donation Steatosis and Defatting Strategies

LT is widely considered a treatment modality for end-stage liver diseases. In countries with limited availability of cadaveric donations, LDLT mitigates organ shortages and reduces mortality rates among transplant candidates.[17] Graft selection is a critical factor for achieving favorable post-transplant outcomes. Donor liver steatosis is considered a risk factor for poor outcomes after transplantation, as it increases the risk of primary non-function (PNF).[14] Macrosteatosis exceeding 30% in donors is an independent risk factor for graft failure.[15,16] The use of liver grafts with mild steatosis (<30% macro- and microsteatosis) is generally accepted to not be related with an elevated risk of PNF.

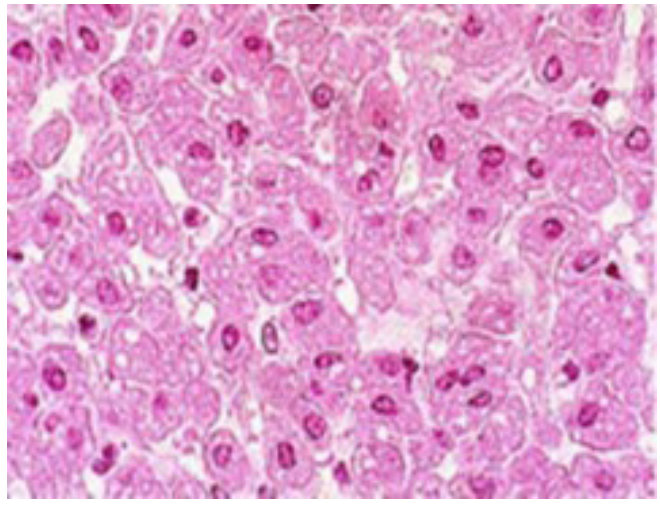

Figure 2. Microvesicular steatosis.

Table 1. Etiology of Macro- and Microvesicular Steatosis

Macrovesicular Steatosis	Microvesicular Steatosis
Obesity	Reye's syndrome
Malnutrition	Viral infections
Metabolic Disorder (Wilson's disease)	Acute Fatty Liver of Pregnancy
Infectious Diseases (e.g., Hepatitis C)	

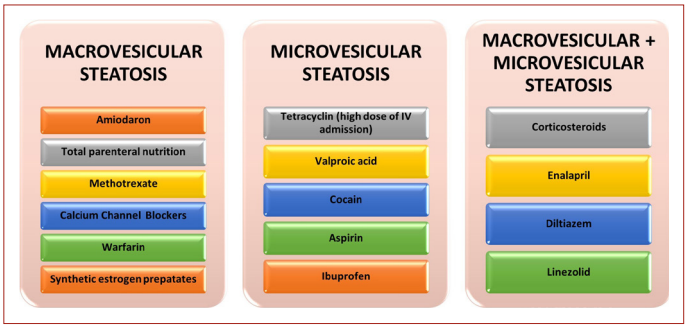

Figure 3. Drugs Causing Macrovesicular and/or Microvesicular Steatosis.

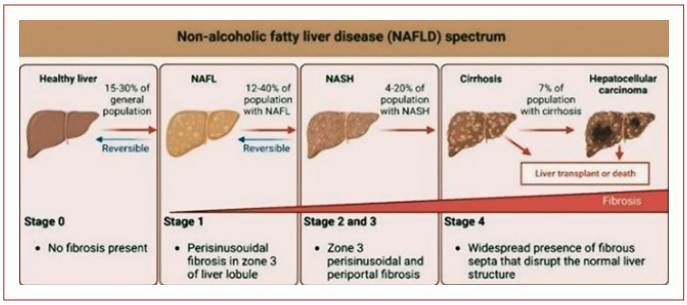

Figure 4. Stages of NAFLD progression from a healthy liver to HCC. *NAFL: Non-alcoholic fatty liver; NASH: Non-alcoholic steatohepatitis.*

Contrary to the livers with mild steatosis, the use of grafts with moderate macrosteatosis (30–60%) is still debated and often regarded as a relative contraindication.[17] Severe macrosteatosis (>60%) is considered an absolute contraindication for LT and is strongly associated with renal and hepatic failure in graft recipients.[18–20] Currently, there are no definitive guidelines on the use of steatotic donor livers, and decisions largely rely on the judgment of the transplant physician or surgeon.[17]

The reduction of lipid content in steatotic grafts, known as defatting, increases access to a larger donor pool and has the potential to alleviate organ shortages. There is growing interest in developing defatting strategies to rapidly reduce graft steatosis before LT and improve results in transplants involving steatotic grafts. Dietary interventions, exercise, and pharmacological treatments are utilized to address steatosis in living donors. Strategies for reducing liver fat in potential donors are summarized in Table 2.[6]

At İnönü University's Liver Transplantation Institute, a defatting protocol is applied to patients exhibiting predonation steatosis. This protocol includes an 8-week protein-rich diet (1000 kcal/day), 4 weeks of fenofibrate (267 mg/day), 8 weeks of exercise (600 kcal/day), 8 weeks of L-Carnitine (300 mg/kg/day), 8 weeks of essential phospholipid therapy (phosphatidylcholine 1500 mg/day), 8 weeks of Vitamin E supplementation (800 IU/day), and 8 weeks of Omega-3 supplementation 1000 mg/day, consisting of 500 mg docosahexaenoic acid (DHA) and 150 mg eicosapentaenoic acid (EPA).

Table 2. Defatting Strategies in Living Liver Donors

First Author	Year	Number of Donors Treated	Number of Transplantations Performed	Applied Strategy	Treatment Duration	Outcome
Nakamuta et al.[21]	2015	11	7	High-protein diet (1000 kcal/day) + exercise (600 kcal/day) + bezafibrate (400 mg/day)	2-8 weeks	Significant improvement in macrovesicular steatosis and a remarkable decrease in body weight and BMI
Clavien et al.[22]	2010	42	3	Omega-3 fatty acids (1 g/day)	12 months	Improvements in biochemical, ultrasonographic, and hemodynamic features of liver steatosis with n-3 PUFA supplementation
Fujii et al.[23]	2015-2019	8	8	Daily caloric intake < 1600 kcal/day + exercise (20 minutes, three times per week) + statin therapy for patients with hyperlipidemia +/- essential phospholipid therapy (1500 mg/day) for patients unresponsive to treatment	5-16 weeks	No major complications observed post-donor hepatectomy; 100% graft and patient survival at three months
Doyle et al.[24]	2016	16	14	OptiFast administered to 16 donors (53 donors received no intervention); patients were provided with OptiFast 900 meal-replacement shakes containing 225 kcal, 22.5 g protein, 7.5 g fat, and 16.8 g carbohydrates per portion. Four shakes were consumed daily.	4 weeks	Reduction in the prevalence of <10% macrosteatosis among OptiFast-treated patients; greater BMI reduction in the OptiFast group compared to untreated individuals

2.2. Post-Hepatectomy Steatosis and Defatting Strategies

It is widely recognized that the regenerating liver temporarily accumulates lipids. Numerous experimental reports demonstrated that acute hepatic steatosis is necessary for normal liver regeneration.[29]

Glucocorticoid levels increase because of preoperative and postoperative fasting and surgical stress after liver resection, leading to a reduced rate of glucose utilization. Therefore, the primary energy source for liver regeneration - an energy-intensive process - is derived from fatty acids released into the bloodstream through lipolysis. Increased fatty acid uptake by hepatocytes results in transient steatosis, a critical event during liver regeneration.[25,26] Hepatic lipid accumulation during liver regeneration peaks within 12–24 hours after liver hepatectomy, and triglyceride content increases three- to fourfold when compared to preoperative levels. Lipid levels gradually return to baseline by 72 hours postoperatively.[27,28]

While large lipid reserves can provide massive energy for liver regeneration, excessive lipid accumulation and lipid peroxidation may induce hepatocyte apoptosis, trigger aseptic inflammation, and impair liver function.[34]

In a study carried out in 2023, Xi et al. examined the role of L-carnitine in liver regeneration, highlighting its ability to support lipid metabolism. The authors reported that L-carnitine promotes cellular regeneration in the liver by enhancing lipid metabolism and reducing aseptic inflammation due to excessive lipid accumulation.[29]

Oral administration of omega-3 fatty acids is related with improvements in steatosis and liver regeneration in rats subjected to a methionine-choline-deficient diet, as well as enhanced functional recovery following partial hepatectomy.[30]

For healthy individuals, the recommended dose of L-carnitine is 15 g/day for healthy individuals and ranges between 100 and 400 mg/kg/day for patients with carnitine deficiency.[31,32] Studies indicated that high doses of L-carnitine supplementation may cause certain side effects, including gastrointestinal issues, diarrhea, and the production of trimethylamine, which leads to a fishy odor.[33] In a meta-analysis investigating the efficacy and safety of carnitine supplementation in patients with NAFLD, it was found that high therapeutic doses (>1000 mg/day) and prolonged treatment durations (>24 weeks) could provide benefits without causing significant adverse effects.[34]

The three most clinically significant omega-3 polyunsaturated fatty acids (PUFAs) are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Deciding which omega-3 PUFA to use in the treatment of NAFLD is an important point. Preclinical and clinical studies revealed that EPA and DHA do not have equivalent effects on NAFLD, with DHA being superior to EPA in controlling steatosis, inflammation, and fibrosis.[35] When examining four meta-analyses of randomized controlled trials on omega-3 PUFA treatment in NAFLD patients, the administered omega-3 PUFA doses varied between 0.83 and 6.4 g/day among 561 patients, with treatment durations spanning 2 to 18 months.[36–39]

3. Steatosis and Its Management in Recipients After LDLT

There are two forms of liver steatosis with distinct histological features and prognoses after LT. The recurrent or de novo steatosis forms observed post-transplantation are becoming an increasing concern among LT recipients. De novo steatosis refers to the development of steatosis after LT in patients with no prior diagnosis of steatosis. Recurrent steatosis, on the other hand, occurs when steatosis reappears post-transplantation in patients who had been diagnosed with steatosis before LT.[40] In a study carried out in 2014 by Melanie et al. comparing the clinical, biological, and histological characteristics of recurrent and de novo steatosis post-transplantation, recurrent steatosis was found to have an earlier onset, greater severity, and to be an irreversible condition.[41]

It is very important to understand and evaluate the risk factors for post-LT steatosis. Risk factors for de novo and recurrent steatosis are summarized in Table 3.[40,42]

3.1 Management of NAFLD Following LT

At this moment, there is no pharmacological therapy approved by any health authority for NAFLD treatment. NAFLD management after LT is extrapolated from the management of non-LT NAFLD. It largely relies on lifestyle modifications and optimization of metabolic and medical comorbidities.[43] The management strategy for post-LT NAFLD is summarized in Figure 5.[43] Given the data obtained from non-transplanted patients, the medications used in NAFLD/NASH treatment are summarized in Figure 6.[42]

At Inonu University's Liver Transplantation Institute, a defatting protocol is applied for post-transplant patients exhibiting steatosis. This protocol consists of an 8-week high-protein diet (1000 kcal/day), L-Carnitine (300 mg/kg/day), comorbidity management, immunosuppression management, 8 weeks of essential phospholipid therapy (phosphatidylcholine 1500 mg/day), 8 weeks of Vitamin E supplementation (800 IU/day), and 8 weeks of Omega-3 supplementation (1000 mg/day, consisting of 500 mg DHA and 150 mg EPA).

Table 3. Risk Factors for De Novo and Recurrent Steatosis

Pre-transplantation Risk Factors	Post-transplantation Risk Factors
Presence of cardiometabolic comorbidities	Weight gain
Pre-transplant obesity	Sarcopenic obesity
PNPLA3 and TM6SF2 gene polymorphisms	Use of immunosuppressive medications (extended steroid therapy, calcineurin inhibitors, sirolimus)
Pre-existing NASH and/or alcoholic liver disease and/or hepatitis C virus infection	Advanced age
	Renal dysfunction
	Sleep apnea
	Donor steatosis
	Metabolic syndrome (diabetes, hypertension, dyslipidemia)

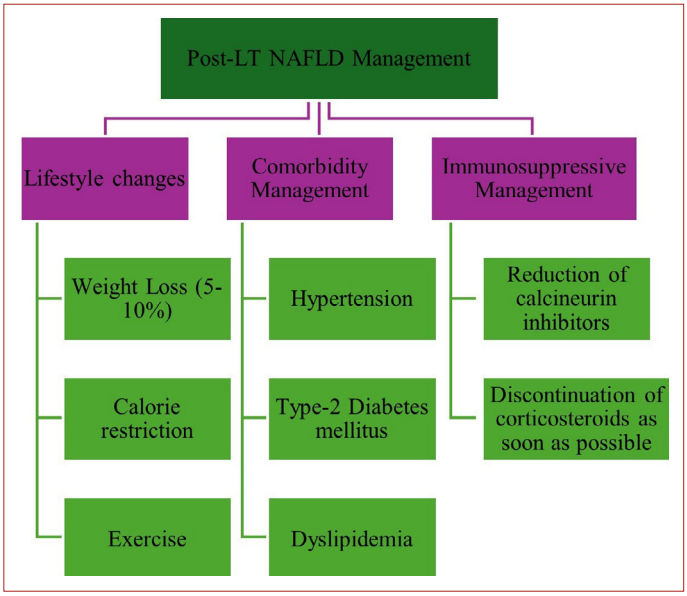

Figure 5. Management of NAFLD After LT.

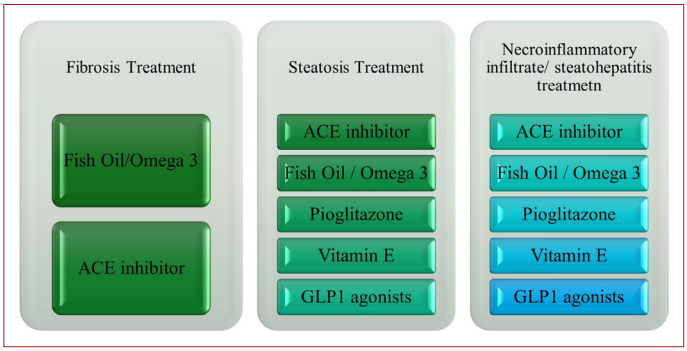

Figure 6. Medications Used in NAFLD/NASH Treatment.

3.1.1 Post-Transplant Immunosuppression Management

Immunosuppressive therapy is very important for improving allograft survival and outcomes but is associated with numerous side effects, including altered metabolic homeostasis.[43] The development of metabolic risk factors related with immunosuppressive regimens can jeopardize long-term graft function and survival.[44] In patients with recurrent or de novo NAFLD/NASH after LT, modifications in the immunosuppressive regimen are necessary to optimize the management of metabolic comorbidities.

Steroid use poses a significant risk for the development of post-transplant diabetes mellitus (PTDM) and other features of metabolic syndrome. Therefore, it should be minimized and/or discontinued approximately three months post-transplant, whenever feasible. Calcineurin inhibitors (CNIs) are closely associated with hypertension, diabetes, and hyperlipidemia. For patients who fail to achieve adequate control of these comorbidities despite medical treatment, dose reduction should be considered. Mammalian target of rapamycin (mTOR) inhibitors are significantly associated with hyperlipidemia. For patients in whom hyperlipidemia remains uncontrolled, transitioning to an alternative immunosuppressive agent is recommended.[43]

3.1.2. Post-Transplant Hypertension Management

Arterial hypertension is seen in 30–50% of transplant recipients and has a prevalence of approximately 70% during long-term follow-up.[45] Hypertension is multifactorial in origin but is closely associated with the use of CNIs and glucocorticoids. For liver transplant recipients, the target blood pressure should be <130/80 mmHg to decrease the risk of cardiovascular disease.[46] Achieving this blood pressure target often requires specific pharmacological therapy. In patients without proteinuria, dihydropyridine calcium channel blockers (DCCBs) are the first-line option. Amlodipine, felodipine, and nicardipine are preferred as first-line agents due to their long half-life, minimal interactions with CNIs, and limited side effects.[47]

Approximately 30% of patients necessitate multiple agents for effective BP control. If CCBs are ineffective or poorly tolerated, adding or substituting a cardioselective beta-blocker, such as metoprolol or atenolol, is recommended. Non-selective beta-blockers are avoided due to their impact on portal blood flow. In patients with difficult-to-control hypertension and/or diabetes, angiotensin-converting

enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs) are preferred.[47] In their study carried out in 2023, Ahad et al. investigated the impact of ARB use on liver steatosis in transplant recipients, reporting a significant association between ARB use and reduced incidence of hepatic steatosis.[48]

3.1.3. Post-Transplant Diabetes Mellitus Management

The prevalence of PTDM ranges between 31% and 38%, with a new-onset diabetes prevalence of 13% to 28% during the first three years after surgery.[49] PTDM severely impacts the prognosis of transplant recipients, increasing 10-year mortality, infection rates, and cardiovascular events. Considering these adverse effects on post-transplant outcomes, maintaining euglycemia is a primary goal in the management of transplant recipients. Male gender, ethnicity, family history, and hepatitis C are well-established risk factors for the development of PTDM. Once diagnosed, PTDM requires the evaluation of specific therapeutic strategies. Lifestyle modifications, representing the first-line approach, have generally been insufficient for achieving adequate glycemic control.[50]

Among oral antidiabetic agents, administration of metformin, pioglitazone, and sulfonylureas after solid organ transplantation (SOT) was studied and demonstrated to be safe when used alone or in combination with insulin.[51,52] Recently, dipeptidyl peptidase-4 (DPP-4) inhibitors started to be routinely administered to SOT recipients due to their beneficial effects on weight loss. However, the potential impact of DPP-4 inhibitors and glucagon-like peptide-1 (GLP-1) receptor agonists on the bioavailability of immunosuppressive drugs remains unclear. Specific drug-drug interactions, such as those between sitagliptin and cyclosporine or vildagliptin and tacrolimus, warrant further investigation.[53] While data on sodium-glucose cotransporter-2 (SGLT2) inhibitors in transplant recipients are currently lacking, recent studies suggest that empagliflozin decreases liver steatosis and improves alanine aminotransferase levels in patients with type 2 diabetes and NAFLD.[53] Insulin continues to be the preferred therapy when therapeutic targets are not achieved, or metabolic homeostasis cannot be maintained.[54]

3.1.4. Post-Transplantation Management of Dyslipidemia

The prevalence of dyslipidemia after LT ranges between 45% and 71%. Immunosuppressive therapy, DM, obesity, and the genetic characteristics of the recipient represent the primary risk factors for post-LT dyslipidemia.[50,55] Compared to the pre-transplant period, dyslipidemia developing after LT is often resistant to dietary interventions, necessitating pharmacological treatment. The European Society of Cardiology recently proposed stringent targets for the management of dyslipidemia in solid organ transplant (SOT) recipients, aligned with those recommended for patients at high and very high cardiovascular risk.[56] Statins are considered the first-line therapy in LT recipients, but potential interactions with immunosuppressive medications should be closely monitored.[57] Statins such as fluvastatin, pravastatin, pitavastatin, and rosuvastatin, which are metabolized via different cytochrome P450 enzymes, are associated with fewer pharmacological interactions.[58] In LT recipients who cannot tolerate statins, ezetimibe may be considered as an alternative.[59]

Conclusion

The efficacy of defatting strategies has been demonstrated in only a limited number of studies, underscoring the need for more comprehensive research evaluating defatting agents and protocols. To date, there is no approved pharmacological treatment for NAFLD. Managing NAFLD in LT recipients requires a multidisciplinary and holistic approach, heavily reliant on lifestyle modifications, optimization of metabolic and medical comorbidities, and the individualization of immunosuppressive therapy.

Disclosures

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Original Research

Early Results of Hepatitis B Recurrence After Postoperative Close Monitoring of Patients Who Have Undergone Liver Transplantation for Hepatitis B

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Abstract

Objectives: Hepatitis B virus (HBV) is a a frequent cause of liver cirrhosis and hepatocellular carcinoma (HCC), that requires liver transplantation. This study aims to analyze HBV recurrence rates in the early postoperative period. Our second goal was to identify contributing factors of HBV recurrence following liver transplantation.

Methods: This retrospective study included 54 patients who underwent liver transplantation for HBV-related liver diseases at Inonu University Liver Transplant Institute in 2024. Data on demographics, preoperative viral markers, postoperative anti-HBs levels, and immunoglobulin and antiviral therapy regimens were collected from each patient. Statistical analyses were performed to compare outcomes based on risk stratification and anti-HBs levels.

Results: Fifty-four patients (81.48% male; mean age: 52.13±10.54 years were included in the study. In total 1.9% (n=1) experienced HBV recurrence during the early postoperative period. The mean Model for End-Stage Liver Disease (MELD) score was 18.46±6.15, and HBV DNA was negative in 81.48% of patients in the pretransplant period. Anti-HBs titers exceeded 100 IU/L in 79.63% of patients on postoperative day 7. There were no notable differences in demographic or clinical variables between patients with anti-HBs titers exceeding 100 IU/L and those with levels below this threshold.

Conclusion: The combination of hepatitis B immunoglobulin (HBIG) and antiviral therapy effectively prevents HBV recurrence following liver transplantation . Maintaining anti-HBs titers above 100 IU/L is critical. Further studies are needed to optimize prophylactic strategies to improve the outcomes in patients transplanted for HBV-related liver disease.

Keywords: Hepatitis B immunoglobulin, HBV recurrence post-liver transplantation, HBV-related liver diseases

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Globally, hepatitis B virus (HBV) remains one of the most prevalent viral infections and is a frequent cause of liver cirrhosis and primary indication for liver transplantation in Türkiye. Chronic HBV infection not only leads to liver failure but also increases the risk of hepatocellular carcinoma (HCC) in the individuals . Before the development of effective prophylactic strategies, HBV recurrence following liver transplantation

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and immunosuppression was considered unavoidable. Frequently, these recurrences rapidly advanced to graft failure that resembling fulminant hepatitis. These observations emphasize the critical role of effective HBV prophylaxis and treatment protocols in achieving successful liver transplantation outcomes. The introduction of prophylactic therapies including the combination of anti-hepatitis B immunoglobulin (HBIG) and potent antiviral agents has revolutionized the prevention of HBV recurrences in liver transplant recipients. These therapeutic and prophylactic measures have significantly reduced HBV recurrence rates, improving graft and overall survival. Despite necessary precautions, HBV recurrence is can be observed in some cases which raises questions about the limitations of current preventive strategies.[1, 2] The effectiveness of antiviral and HBIG therapies has led to generally superior liver transplantation outcomes for HBV-related liver disease compared to other etiologies.[1, 2] However, persistent or recurrent HBV infection remains a critical concern, particularly because it is closely associated with post-transplant HCC recurrence, significantly complicating clinical management.[3, 4]

The challenges of managing HBV in the context of liver transplantation extend beyond recurrence prevention. HBV-related HCC requires strict oncologic surveillance and antiviral therapy to prevent HBV recurrence and subsequent HCC recurrence after liver transplantation. Factors such as pre-transplant viral load, adherence to prophylactic regimens, and potential drug resistance requires continuous evaluation and refinement of the treatment protocols.[4] Evoluating the efficacy of prophylactic therapies and assessing risk factors for recurrence are important for preventing HBV recurrence after liver transplantation.. In this study, we aimed to investigate early postoperative HBV recurrence rates. Furthermore, we aimed to evaluate the underlying factors contributing to recurrence in patients who underwent liver transplantation for HBV-related cirrhosis and HCC in 2024. We hope to provide insights into optimizing prophylactic strategies and improving post-transplant care for HBV-infected patients.

Methods

This study included patients who underwent liver transplantation at our institute in 2024 due to liver diseases associated with HBV infection. The analyzed data included demographic details, MELD scores, transplant dates, and preoperative ELISA test results, which encompassed HBsAg, HBeAg, HBcAg, anti-HBc, anti-HBs, delta Ag, delta Ab, HBV DNA, and HDV RNA. Postoperative data such as administered immunoglobulin doses, initial HBsAg levels, and follow-up HBsAg levels were also analyzed.This study was approved by the Inonu University Scientific Research Publication Ethics Board (approval number: 2024/6685).

We performed a risk stratification to taper the protocol for immunoglobulin administration. Several factors influenced risk stratification for HBV recurrence after liver transplantation. Patients with pre-transplant HBV-DNA, HBe antigen positivity, HIV or HDV coinfection, antiviral non-compliance or resistance, or hepatocellular carcinoma were considered high-risk. Otherwise, they were considered to have low-risk. High-risk patients received an intraoperative dose of 10,000 IU of immunoglobulin. Low-risk patients received 5,000 IU. Both groups then received a daily dose of 2,000 IU for the first postoperative week. The goal of this therapy was to maintain an anti-HBs antibody titer of at least 100 IU/mL. If, at the end of the first postoperative week, antibody titers were below this target, immunoglobulin therapy was continued for another week in both groups. In 2024, our institute performed a total of 257 liver transplants, with 54 of these were due to HBV-related liver diseases. HBV recurrence was observed in only one patient, indicating a recurrence rate of 1.9% with the prophylaxis protocol that used.

Statistical Analysis

Categorical variables were and expressed as number of affected individuals and th percentage of the study population (percentages), while continuous variables were tested for normality using the Shapiro-Wilk test. Continuous data were expressed as median (minimum-maximum) and mean±standard deviation. Comparisons of categorical variables between groups were performed using Fisher's exact chi-square test. For continuous variables, comparisons between two independent groups were performed using the Mann-Whitney U test. A p-value of <0.05 was considered as statistically significant. All statistical analyses were performed using IBM SPSS Statistics 26.0 for Windows (New York, USA).

Results

In total 54 patients included in this study. Fourty-four (81.48%) were male and 10 (18.52%) were female. The mean age of the patients was 52.13±10.54 years (median: 54, range: 20-72). Of these patients, 28 (51.85%) had HBV-related liver disease, while 26 (48.15%) were diagnosed with HBV+HCC. The mean MELD score was 18.46±6.15 (median: 17, range: 6-32), and the mean body mass index (BMI) was 26.93±5.33 kg/m^2 (median: 25.86, range: 18.94-40.86). HBV DNA was negative in 44 patients (81.48%) and positive in 10 patients (18.52%). HDV RNA was negative in 39 patients (72.22%) and positive in 15 patients (27.78%). On

postoperative 7th day , anti-HBs levels exceeded 100 IU/L in 43 patients (79.63%) and were below 100 IU/L in 11 patients (20.37 %). HBV recurrence was observed in only one patient (1.85%) during our follow-up period. The mean survival time was 128.04±89.85 days (median: 114, range: 10-296). The clinical and demographic data are summarized in Table 1.

Patients with postoperative day-7 anti-HBs levels below and above 100 were compared based on their demographic and clinical characteristics. there were no statistically significant differences between the two groups in terms of age (p=0.581), recipient height (p=0.755), recipient weight (p=0.874), BMI (p=0.632), MELD score (p=0.274), or survival duration (p=0.499). Similarly, there was no significant difference between the groups in terms of gender distribution (p=1.0), diagnosis distribution (HBV and HBV+HCC) (p=0.224), HBV DNA status (p=0.408), or HDV RNA status (p=1.0). These findings suggest demographic and cinical variables have no effect on the postoperative day-7 anti-HBs antibody titers. The results of our evaluation are summarized in Table 2.

Table 1. Demographic and Clinical Characteristics of the patients included in the study.

Variables	n	%	Mean±SD
Gender (Female/Male)	10/44	18.52/81.48	
Diagnosis			
HBV	28	51.85	
HBV+HCC	26	48.15	
HBV DNA			
Negative	44	81.48	
Positive	10	18.52	
HDV RNA			
Negative	39	72.22	
Positive	15	27.78	
Anti-HBs Day 7			
Below 100	11	20.37	
Above 100	43	79.63	
HBsAg Recurrence			
Negative	53	98.15	
Positive	1	1.85	
Age (Years)			52.13±10.54
Recipient Height (m)			1.71±0.09
Recipient Weight (kg)			78.39±15.27
BMI (kg/m^2)			26.93±5.33
Survival (Days)			128.04±89.85
MELD Score			18.46±6.15

SD: Standard Deviation; Min: Minimum; Max: Maximum; HBV: Hepatitis B; HCC: Hepatocellular Carcinoma; BMI: Body Mass Index; MELD: Model for End-Stage Liver Disease.

Discussion

In our study, the recurrence rate of hepatitis B virus (HBV) after liver transplantation in patients with HBV-related liver disease was remarkably low (1.92%, n=1). This finding emphasizes the effectiveness combination of hepatitis B immunoglobulin (HBIG) and antiviral agents to prevent HBV recurrence post-transplantation. Prophylactic HBV therapy has been shown to effectively reduce HBV recurrence after liver transplantation, thereby enhancing the positive impact of immunosuppression management in the post-transplant period.There are studies that report HBV recurrence rates less than 5% following prophylactic therapy with HBIG and antiviral agents.[7] For instance, a the study by Roche et al.,[8] a combination of immunoglobulin and antiviral agents reduced the recurrence rate of HBV to as low as 3.5%, effectively. These findings are consistent with our study, suggesting that the combination of these prophylactic measures is highly effective in reducing the recurrence of HBV in transplant recipients.Most of the patients who did not experience HBV recurrence (82.35%) had anti-HBs levels over 100 IU/L on postoperative 7th day. Our observation is supported by previous research suggesting that high anti-HBs titers play a key protective role in preventing HBV recurrence.[9] Higher levels of anti-HBs are often associated with better immunological control of the virus and reduced risk of reinfection, making it a critical marker for predicting the success of prophylactic therapy. The findings of our study emphasize the importance of maintaining high anti-HBs antibody titers in the immediate postoperative period. However, it is noteworthy that even in patients with anti-HBs antibody titers below 100 IU/L, HBV recurrence rates remained low (p>0.05). This observation suggests that factors other than anti-HBs antibody titters may play a role in the prevention of HBV recurrence. The role of immunosuppression treatment, the patient's immune response, and the genetic or viral factors that influence the susceptibility to HBV reinfection remain areas that need further research. It is well documented that the immune status of the patient, as well as the degree of immunosuppression, have a significant impact on the outcome of post-transplant HBV management.[10]

Additionally, the analysis of demographic and clinical variables, such as age, gender, BMI, MELD score, and liver function, revealed no significant differences between the groups with different anti-HBs antibody titter on postoperative day-7 (p>0.05). This suggests that factors such as age, sex, and MELD score did not affect anti-HBs antibody titters in the early post-transplant period. These findings are valuable as they help highlight the factors that have various influences on the success of prophylactic treatment. It is particularly

Table 2. Comparison of Demographic and Clinical Characteristics Based on Anti-HBS Level

Variables	Anti HBS 7. Day		p
	<100 ıu/ml	>100 ıu/ml	
Age	50.55±14.81	52.53±9.34	0.581*
Recipient Height	1.7 (1.6-1.82)	1.72(1.5-1.85)	0.755**
Recipient Weight	77.73±15.94	78.56±15.28	0.874*
BMI	26.23±6.16	27.11±5.16	0.632*
MELD Score	16.64±5.03	18.93±6.37	0.274*
Survival Duration (Days)	157(11-287)	114(10-296)	0.499**

Variables are presented as mean±standard deviation or median (minimum-maximum) according to the normality of the distribution.

	n (%)	n (%)	p
Gender			
Female	2 (18.18)	8 (18.60)	1.0****
Man	9 (81.82)	35 (81.40)	
Diagnosis			
HBV	8 (72.73)	20 (46.51)	0.224***
HBV+HCC	3 (27.27)	23 (53.49)	
HBV DNA			
Negative	8 (72.73)	36 (83.72)	0.408****
Positive	3 (27.27)	7 (16.28)	
HDV RNA			
Negative	8 (72.73)	31 (72.09)	1.0****
Positive	3 (27.27)	12 (27.91)	

*: Mann Whitney U test; **: Independent sample t-test; ***: Yates's correction chi-square test; ****: Fisher's exact chi square; BMI: Body mass Index; MELD: The Model for End-Stage Liver Disease.

noteworthy that even in patients with HDV co-infection, the recurrence rates of HBV remained low. This emphasizes the fact that prophylactic treatment including combination of immunoglobulin and antiviral agents is effective even in the presence of HDV, which is known to complicate HBV infection and may alter its clinical course.[11] The importance of this lies in the fact that HDV coinfection is associated with a more severe clinical course of HBV infection, including a higher incidence of liver failure, thus posing greater challenges for disease management.The demonstrated efficacy of prophylactic therapies in these cases affirms the robustness of current therapeutic strategies.

Maintaining anti-HBs antibody titters above a protective threshold in most patients (above 100 IU/L) supports the surveillance strategy that is essential to ensure the success of the transplant. However, the study also suggests that more individualized approaches may be beneficial in further reducing recurrence rates. This may involve tailoring immunosuppression therapies and antiviral regimens based on the patient's baseline immunological status, viral load, and other genetic or environmental factors that influence the immune response.

The results of our study highlight the need for long-term follow-up to understand the persistence of HBV control and the long-term risks of recurrence. While the early results in this cohort are promising, ongoing surveillance of HBV DNA, anti-HBs levels, and liver function will be crucial in assessing the durability of the treatment and in identifying any delayed recurrences of HBV infection. Future studies are need that incorporates more diverse patient populations, longer follow-up periods, and the evaluation of alternative treatment regimens, including newer antiviral agents or personalized immunosuppression strategies, to optimize the prevention of HBV recurrence. Limitations of the study include patients who received liver transplantation after 2024, patients who received liver transplantation only due to hepatitis B, and only adult patients.

In conclusion, the results of this study demonstrate that the combination of HBIG and antiviral therapy can effectively control HBV recurrences in the post-transplant period. We observed low recurrence rates in the early postoperative period. These findings support the current prophylactic protocols for HBV management in liver transplant patients and suggest that further refinements and personalized

treatment strategies may hold the potential reduce the recurrence rates further. However, multi-center studies with high patient volumes and long-term follow-up are needed to confirm the findings and to optimize management strategies for patients with HBV-related liver disease undergoing transplantation.

Disclosures

Ethics Committee Approval: This study was approved by the Inonu University Scientific Research Publication Ethics Board (approval number: 2024/6685).

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Conflict of Interest: None declared.

Authorship Contributions: Concept – D.Y.B.; Design – D.Y.B.; Supervision – S.Y.; Materials – I.B.C.; Data collection &/or processing – D.Y.B.; Analysis and/or interpretation – I.B.C.; Literature search – M.H.; Writing – D.Y.B.; Critical review – S.Y.

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Original Research

Identify of Potential Genetic Biomarkers for Hepatitis C Virus Related Hepatocellular Carcinoma

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Abstract

Objectives: Hepatocellular carcinoma (HCC) is a considerable global health concern. This study attempts to analyze gene expression data between liver tissues with HCV-related HCC and healthy liver tissues to identify potential biomarkers that contribute to HCC development.

Methods: We analyzed RNA sequencing data from liver tissues with HCV-related HCC and healthy liver tissues in this study. We retrieved the dataset from NCBI using the code GSE140845. We performed gene expression analysis using the Limma software package in the R programming language, defining genes as differentially expressed if they had a log2 fold change (log2FC) > 1 and a p > 0.05. We conducted data visualization using scatter plots, UMAP, volcano plots, and mean difference (MD) plots.

Results: A total of 20,868 genes were analyzed between the HCV-HCC and healthy liver tissue groups, and 3,303 genes were found to be significantly differentially expressed. Genes such as AKR1B10, MUC13, SLC22A11, and SPINK1 showed upregulation in the HCV-HCC group, whereas CNDP1, IGFALS, PVALB, and CLEC4M showed downregulation. These genes have the potential to serve as biomarkers and play critical roles in understanding the mechanisms of HCC development.

Conclusion: This study highlights the differential regulation of genes associated with HCV-HCC, emphasizing their potential roles in the pathogenesis of HCC. Notably, the identified biomarkers hold promise as therapeutic targets. These findings may contribute to personalized medicine approaches and enable the development of novel strategies for the prevention and treatment of HCC.

Keywords: HCC, HCV, HCV-related HCC, Gene expression analysis, Biomarkers

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Globally, hepatocellular carcinoma (HCC), the primary form of liver cancer, is the fourth leading cause of cancer-related death, responsible for over 800,000 deaths in 2017 alone. As such, it poses a serious threat to global health.[1, 2] The prevalence of liver cancer varies greatly, with East Asia having the highest rates, which can be more than five times higher than in places like South Asia and Eastern Europe.[1] This geographical disparity is mostly due to the high incidence of viral hepatitis infections, including hepatitis B virus (HBV) and hepatitis C virus (HCV) cause a worldwide fatality rate of 76%.[3] Vaccination progress in immunization and treatment methodologies, the prevalence of liver cancer continues to be significant, especially in low- and middle-income nations with restricted healthcare access.[3, 4]

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HCV, a hepatotropic RNA virus, is a bloodborne infection that only impacts the liver. The majority of patients infected with HCV do not spontaneously eradicate the infection, resulting in a chronic condition. Chronic hepatitis C virus (HCV) infection can progress to severe liver diseases, including fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). While approximately 71 million people are currently infected with HCV, only 20–30% develop cirrhosis, and 1–4% of cirrhotic patients progress to HCC annually.[5, 6] HCV infection constitutes a burgeoning worldwide health concern. HCV is prevalent in many countries and is progressively exerting significant pressure on society and healthcare systems. Chronic sequelae, such as cirrhosis and HCC, are increasingly common.[7] The natural history of chronic HCV infection is characterized by slow progression, with severe liver disease developing in only a minority of cases within the first 10–15 years post-infection. Therefore, patient age at infection and the duration of chronic infection are key factors influencing morbidity and mortality.[8]

The processes via which HCV leads to HCC are complex, encompassing both direct viral impacts and host genetic influences. The inflammatory environment caused by chronic HCV infection fosters conditions conducive to genetic mutations and genomic instability, which are indicative of cancer progression.[9, 10] Through the induction of oxidative stress and the generation of reactive oxygen species (ROS), HCV can cause DNA damage and subsequent mutations, thereby promoting carcinogenesis.[9, 10] Genomic and epigenomic modifications significantly contribute to HCV-related HCC. Multiple studies have identified specific genetic variations that may influence an individual's susceptibility to HCC in the context of HCV infection. Genetic polymorphisms in CTLA-4 and IL-4 are linked to an increased incidence of HCC in individuals infected with hepatitis C virus.[11, 12]

In summary, the association between HCV and HCC is defined by chronic inflammation, genetic predisposition, and direct viral carcinogenic processes. Comprehending these relationships is essential for formulating tailored prevention and therapy strategies for HCV-related HCC. This study will use gene expression data from HCV-related HCC and normal liver tissues to perform differential expression analyses and evaluate the relationship between HCV and HCC. The goal is to identify genes that exhibit differential regulation in the HCC group. Advanced bioinformatics analysis will support the identification of these genes as biomarkers for HCC, suggesting their role in the disease's development.

Methods

Data Set

To understand the genetic background of HCV-related HCC, this study analyzed microRNA data (RNA sequence data) from normal liver tissue and liver tissue with HCV-related HCC. The data used in this study were obtained from the National Center for Biotechnology Information (NCBI) under accession number GSE140845.

RNA-Sequence Analysis (RNA-Seq)

RNA sequencing (RNA-seq), a well-established and powerful technology, has revolutionized transcriptomics by enabling quantitative and comprehensive analysis of complete transcriptomes. Unlike hybridization-based methods, RNA-seq directly sequences RNA, allowing for the discovery of novel transcripts and precise quantification of known transcripts. This sequencing-based approach offers several advantages, including a broad dynamic range for expression level measurement, reduced background noise leading to highly accurate and reliable data, and precise determination of exon-intron boundaries, single nucleotide polymorphisms (SNPs), and other transcript variants. Due to these advantages, RNA-seq is well-suited for a wide range of discovery-based research projects.[13]

Transcriptomics

The transcriptome represents the complete set of RNA transcripts—including mRNA, tRNA, rRNA, and non-coding RNAs—produced by a cell, tissue, or organism at a specific time. Unlike the relatively stable genome, the transcriptome is highly dynamic and responsive to environmental factors such as pH, nutrient availability, temperature, and intercellular signaling. Fluctuations in gene transcription in response to various cellular processes drive these changes, altering the mRNA composition of the transcriptome. Consequently, the transcriptome provides a snapshot of actively expressed genes within a given context, highlighting the crucial interplay between environment and biological systems.[13, 14]

Transcriptomics is the study of the entire complement of mRNA transcripts generated from a cell's genome, yielding information on their expression profiles. Microarray analysis and next-generation sequencing are widely employed in transcriptomic studies to investigate precise alterations in the transcriptome under specific conditions.[14]

Transcriptomic studies have become increasingly important in elucidating the role of gene expression changes (both up- and downregulation) in complex diseases such as cancer. These studies also provide insights into the interconnectedness of these changes and the underlying biochemical pathways and molecular mechanisms governing cell life cycles and disease progression.[14, 15]

Gene Expression Analysis

Alterations in the physiological state of an organism or cell are invariably reflected in changes in gene expression. Con-

sequently, the assessment of gene expression is of considerable importance in all areas of biological research. DNA microarray technology, which utilizes the hybridization of mRNA molecules to a dense array of immobilized target sequences complementary to specific genes, is one such method employed for gene expression analysis. Investigating the influence of chemical agents on gene expression regulation can provide valuable insights into both functional and toxicological properties. Furthermore, analysis of clinical samples from healthy and diseased individuals can lead to the discovery of novel biomarkers.[16]

Bioinformatics Analysis Phase

Gene expression data from liver tissues with HCV-related HCC and normal liver tissues were analyzed using the limma package in R.[17] Limma, a software suite designed for differential expression analysis using linear models, is applicable to various gene expression technologies, including microarrays, RNA-seq, and qPCR. By employing Empirical Bayes methods, limma provides robust results, particularly with small sample sizes. The analysis generated log2 fold change (log2FC) values, ranking genes by their expression differences. Genes with log2FC > 1 were considered upregulated, while those with log2FC < -1 were considered downregulated.

Data distribution was visualized using box plots. Sample relationships were explored using Uniform Manifold Approximation and Projection (UMAP). Differential gene expression (both up- and downregulated) was visualized using volcano plots, which display the relationship between statistical significance (y-axis) and log2FC (x-axis). In these plots, red indicates upregulated genes, blue indicates downregulated genes, and black indicates genes with no significant change in expression. Mean Difference (MD) plots were also used to visualize differential expression, plotting log2FC against average log2 expression levels. The MD plots used the same color scheme as the volcano plots.

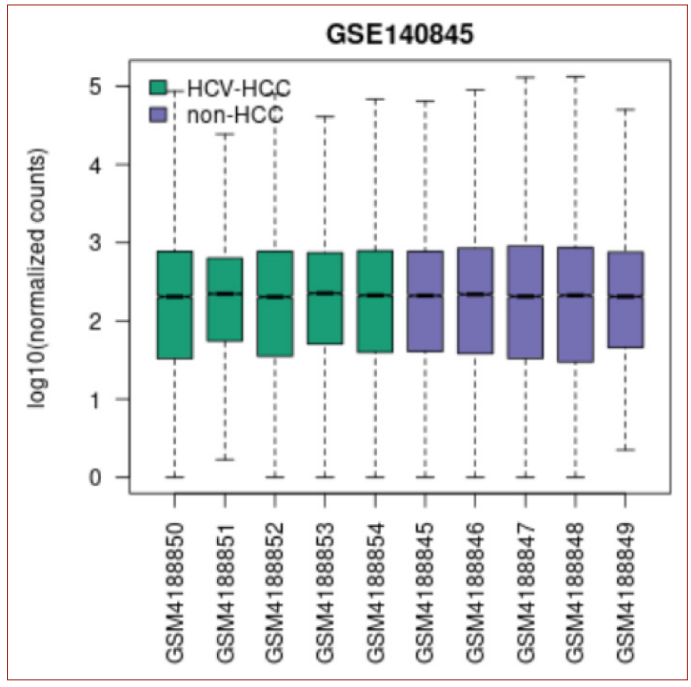

Figure 1. Distribution plot of the samples.

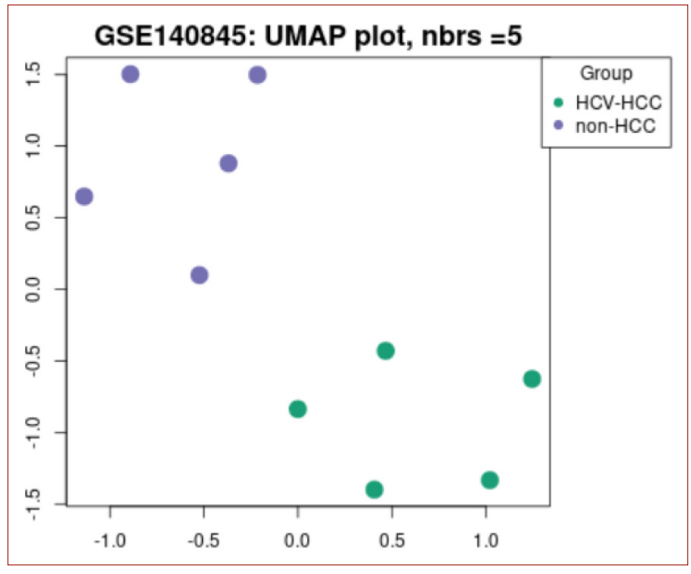

Figure 2. UMAP plot of the samples.

Results

The study includes data from 5 liver tissues with HCV-related HCC and 5 healthy liver tissues. The gender of the 10 individuals was male. The microRNA data were obtained from liver tissues.

Figure 1 shows the scatter plots of the 10 samples used in the study. In the graph, the HCV-HCC symbol is used to represent HCV-related patients and the non-HCC symbol is used to represent healthy liver tissues. The graph was used to show the distribution of values in the samples. A color-coded plot (Fig. 2) was used to assess data normalization before differential expression analysis. The UMAP plot (Fig. 2) demonstrates clear sample clustering based on similar characteristics.

The distribution and clustering pattern of the different groups provide important clues about the biological or molecular similarities of the samples. Green dots represent HCV-HCC tissue samples, while purple dots represent non-HCC tissue samples. This distinction provides a remarkable separation in the classification and grouping of samples and is an effective tool for visualizing different biological states in the dataset.

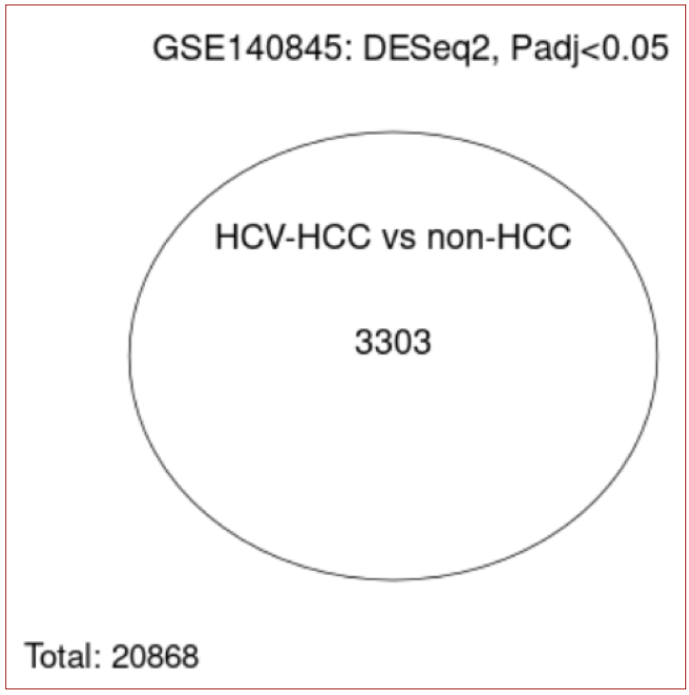

Figure 3. Graph comparing genes between HCV-HCC and non-HCC groups.

Figure 3 represents the comparison of genes between HCV-HCC and non-HCC groups. According to the graph, the total number of genes analyzed is 20868. This represents the number of all genes included in the study. In addition, 3303 genes were statistically significantly differentially expressed between these two groups. The graph was obtained under the condition of |log2FC| > 1.0 (absolute log2 fold change value greater than 1.0) and $p < 0.05$.

Table 1 presents the top 10 upregulated genes identified in the comparison between the two groups. When determining gene expression up-regulation, certain threshold values were taken into account in order to consider statistical significance and biological importance. In this context, the criteria of |log2FC| > 1.0 (absolute log2 fold change value greater than 1.0) and p-value less than 0.05 were used to evaluate the regulation status of genes. These thresholds provide a reliable and standardized approach to determine whether genes show significant differences. The results obtained may contribute to the identification of potential biomarkers by shedding light on the biological significance of genetic differences between groups.

Table 1. Genes up-regulated in HCV-HCC tissues relative to non-HCC

GeneID	padj	pvalue	lfcSE	stat	log2FoldChange	baseMean	Symbol
57016	1,33E-26	3,86E-30	0,725	11,40698	8,26721	5981,23	AKR1B10
56667	2,18E-39	2,11E-43	0,549	13,81374	7,58536	1199,66	MUC13
55867	1,41E-06	2,23E-05	1,509	4,454916	6,72114	480,73	SLC22A11
6424	5,12E-04	2,23E-05	1,425	4,240711	6,042391	325,54	SFRP4
28942	3,29E-12	7,16E-15	1,495	3,987738	5,961361	317,51	IGKV1-8
2719	3,29E-12	7,16E-15	0,766	7,781625	5,96135	1755,83	GPC3
6690	1,41E-06	1,82E-08	1,057	5,628574	5,94965	479,27	SPINK1
60676	2,76E-06	3,93E-08	1,048	5,589756	5,855787	2470,66	PAPPA2
6707	2,76E-06	3,93E-08	1,061	5,494151	5,829656	42,54	SPRR3
441282	2,19E-16	2,01E-19	0,629	9,012934	5,670096	162,67	AKR1B15

Table 2. Genes down-regulated in HCV-HCC tissues relative to non-HCC

GeneID	padj	pvalue	lfcSE	stat	log2FoldChange	baseMean	Symbol
84735	2,47E-28	5,98E-32	0,569	-11,7641	-6,68851	1182,83	CNDP1
730249	4,65E-04	1,97E-05	1,474	-4,2687	-6,29032	378,38	ACOD1
3483	1,29E-40	6,22E-45	0,433	-14,0652	-6,09531	2606,88	IGFALS
5816	8,59E-14	1,24E-16	0,707	-8,27873	-5,85585	26,03	PVALB
10332	3,28E-12	6,97E-15	0,731	-7,78511	-5,68733	931,33	CLEC4M
51266	1,55E-08	1,03E-10	0,88	-6,46222	-5,68658	384,76	CLEC1B
1544	1,32E-11	3,51E-14	0,747	-7,57803	-5,66012	10238,93	CYP1A2
22865	5,71E-09	3,37E-11	0,851	-6,62959	-5,64195	140,16	SLITRK3
339390	9,44E-09	5,93E-11	0,86	-6,54549	-5,63175	940,09	CLEC4G
3781	2,27E-20	1,10E-23	0,561	-10,0325	-5,62507	361,09	KCNN2

Analysis of the dataset identified 10 significantly downregulated genes in the comparison between the two groups (Table 2), using the same log2 fold change threshold (<-1) as for upregulated genes. Differential gene expression between the groups was visualized using volcano plots (Fig. 4) and Mean Difference (MD) plots (Fig. 5). Volcano plots display the relationship between statistical significance and log2 fold change, while MD plots show log2 fold change against mean log2 expression levels. In both plots, red indicates upregulation and blue indicates downregulation, facilitating the identification of genes with significant expression changes. These differentially expressed genes represent potential biomarkers and may play critical roles in disease processes, providing valuable insights for understanding disease mechanisms and identifying therapeutic targets.

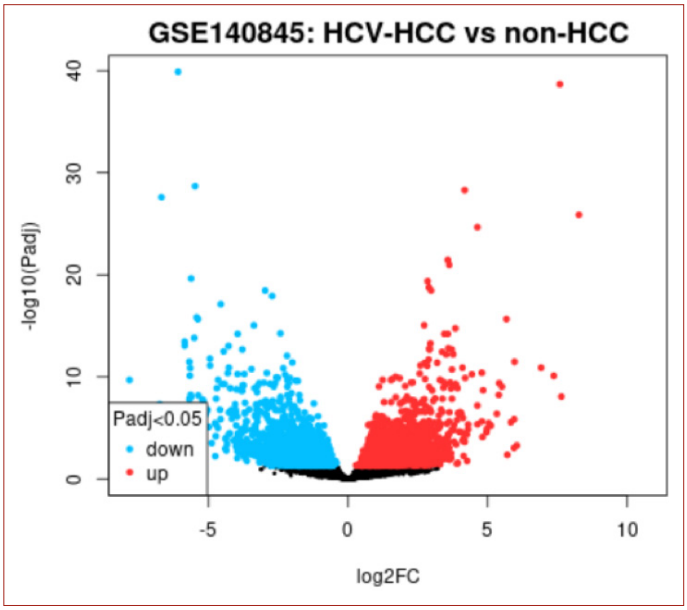

Figure 4. Volcano plot of transcripts in HCV-HCC and non-HCC tissues. (Red dots represent transcripts that increased and black dots represent transcripts whose expression level remained unchanged).

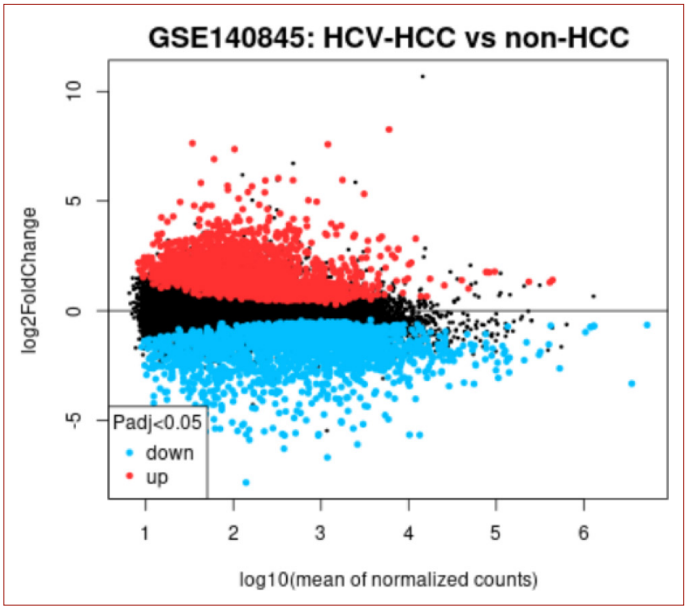

Figure 5. MD plot of transcripts in HCV-HCC and non-HCC tissues (Red dots represent transcripts that increased, and black dots represent transcripts whose expression level remained unchanged).

Discussion

Hepatocellular carcinoma (HCC), a highly aggressive cancer with distinct epidemiological features, remains a major global public health concern due to its substantial economic and health burden.[18-21] Key risk factors include hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholism, metabolic dysfunction-associated steatohepatitis (MASH), and exposure to dietary toxins such as aflatoxins and aristolochic acid, particularly in developed countries. In these cases, HCC often develops following prolonged chronic hepatitis, frequently in the context of HBV- or HCV-related cirrhosis, with an annual incidence of 2–5% in this population.[22] The poor overall survival associated with HCC necessitates improved therapeutic strategies targeting these risk factors to reduce the global disease burden. Consequently, there is growing interest in genomic and molecular research to identify early diagnostic and prognostic markers, elucidate liver carcinogenesis pathways, and develop new therapeutic targets to improve clinical management. Enhanced HCC surveillance is therefore expected to significantly reduce global incidence in the coming decades.[19, 23]

Chronic HCV infection significantly elevates the chance of HCC, with research demonstrating that those with HCV possess a relative risk of 17 for acquiring liver cancer in comparison to the general population.[24, 25] The processes by which HCV facilitates HCC development are complex. HCV infection induces persistent inflammation, potentially resulting in liver fibrosis and cirrhosis, both of which are precursors of HCC.[26] Furthermore, HCV has been demonstrated to elicit diverse molecular alterations in hepatocytes, including the overexpression of certain microRNAs that facilitate viral replication and modify metabolic pathways linked to the etiology of liver disease.[27, 28] In summary, HCV significantly contributes to the global incidence of HCC, with its involvement in liver carcinogenesis underscored by many biological processes and epidemiological data.

This study analyzed gene expression data from HCV-related HCC and normal liver tissues to identify potential biomarkers and understand the genetic basis of HCV-related HCC. As a result of the analyses, many genes were found to be differentially regulated in the HCV-HCC group compared to non-HCC.

Bioinformatic analysis revealed numerous differentially expressed genes, with AKR1B10 showing a 306.55-fold upregulation in the HCV-HCC group compared to the non-HCC group. Similarly, MUC13,SLC22A11, SFRP4, IGKV1-8, GPC3, SPINK1, PAPPA2, SPRR3, AKR1B15 genes had 191.34, 105.41, 65.72, 62.24, 62.24, 61.39, 57.68, 56.49, 50.91 fold up-regulated gene expression, respectively.

The CNDP1 gene was down-regulated 102.53-fold in the HCV-HCC group compared to the non-HCC group. Similarly, ACOD1, IGFALS , PVALB, CLEC4M, CLEC4M, CLEC1B, CYP1A2, SLITRK3, CLEC4G, KCNN2 genes showed 78.24, 68.11, 57.68, 51.26, 51.26, 50.56, 49.86, 49.52, 49.18 fold down-regulation respectively.

Subsequent research on the identified genes holds significant promise, as they may serve as critical biomarkers in the successful treatment of HCC. These biomarkers could revolutionize pharmacological therapies by enabling the design and application of highly targeted treatments. The precise use of genetic biomarkers improves patient care and streamlines healthcare systems, enhancing efficiency. Continued advances in genetic research are expected to expand personalized medicine, transforming modern healthcare.

In conclusion, HCC represents a significant global health burden, and genetic research is indispensable for elucidating its complex pathophysiology. Given that treatment strategies are contingent on the underlying disease etiology, dedicated genetic investigations are essential for the development of targeted preventive and therapeutic strategies. Genetic screening programs for high-risk populations offer opportunities for early detection and timely intervention. Continued progress in genetic research holds great promise for the development of innovative approaches to prevent and manage HCC recurrence, thereby improving patient outcomes.

Disclosures

Ethics Committee Approval: This study utilized a publicly available dataset from the NCBI Gene Expression Omnibus (GEO). As the data were de-identified and publicly available, ethics committee approval was not deemed necessary.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – Z.K., S.A.; Design – Z.K., S.A.; Supervision – S.A.; Materials – Z.K., S.A.; Data collection &/or processing – Z.K., S.A; Analysis and/or interpretation – Z.K., S.A; Literature search – Z.K., S.A; Writing – Z.K., S.A; Critical review – S.A.

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Original Research

A New Approach to Analysis of Clinical Data and Prognostication for Patients with Hepatocellular Carcinoma, Based Upon a Network Phenotyping Strategy (NPS) Computational Method

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Abstract

Objectives: There is a multi-component nature of the influences on HCC progression but integrating them has been difficult. Network phenotyping strategy (NPS) integrates all multi-component relationship facets of HCC progression and aims to lead to a new way of understanding human HCC biology.

Methods: We converted baseline patient demographics, tumor characteristics, blood hematology and liver function test results, consisting of values of 17 standard clinical variables, collected time-coherently at the index visit, into a graph-theoretical data representation.

Results: These data were analyzed by NPS, which processes the patient parameter values together with their complete relationships network. NPS identified 25 disease-progression ordered HCC phenotypes. Clinically relevant NPS results are a) Portal vein thrombosis incidence during HCC progression stratified into 5 narrow ranges; b) NPS identified patients according to aggressive, slow and intermediate tumor growth sub-types; c) Personalized prognostication of mortality was achieved by the 25 NPS phenotypes, independently optimized for respective phenotype sub-cohorts.

Conclusion: The NPS results were implemented as an internet application (https://apkatos.github.io/webpage_nps), where input of 17 clinical parameters provides the patient phenotype, phenotype-characteristic average mortality and personal survival estimate.

Keywords: HCC, Network phenotyping strategy, PVT, survival

Abbreviations: *Network Phenotyping Strategy, NPS; Maximum tumor diameter, MTD; Portal vein thrombosis by tumor, PVT; Time of disease duration at index visit, τ_p; Personal 17-partite graph,$\Gamma(\tau_p)$; Hepatitis, HBV/HCV; serum. α -fetoprotein, AFP; international normalized ratio, INR; alanine transaminase, ALT; aspartate transaminase, AST; alkaline phosphatase, ALKP; γ-glutamyl transferase, GGT; hemoglobin, Hb.*

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There has always been an awareness of the multiple component nature of the various influences on HCC growth (MTD) and invasion (PVT),[1-5] but we have hitherto not been able to integrate them well. Therefore, we used a Network Phenotyping Strategy or NPS,[6] which is designed to analytically process the integrated multi-component relationship facets of HCC seen in patient data, to guide us to a new way of understanding human HCC biology. NPS is derived to identify the stage of HCC disease at its actual "biological" duration during the index visit, at the point when the patient data were collected, by analyzing time-coherent relationship networks between personal clinical variable values. This novel non-statistical processing of extended clinical information in the patient data leads directly to consideration of HCC as a dynamic process, resulting in data-driven objective characterization of HCC stages and eventually, to better personalized outcome predictions.

To achieve this, a new principle of clinical data processing has been formulated. Instead of the standard statistical assumption, that HCC impacts patients more or less similarly, we analyzed HCC dynamics. NPS extracts information about the biological time of patient HCC, encoded in the relationship between coherently observed values of multiple variables. This allows the assigning of patients to objective progression-ordered clinical HCC stages, with earlier stages having good outcomes, whereas later clinical stages have poorer outcomes. This replaces the conventional outcome prognosis using an unknown future clinical state into gaining insight about the stage and prognosis from the known history of the patient's disease, encoded in the known personal biology at the patient index visit, as seen through the clinical variable relationship networks.

To achieve this, a new form of patient data, represented as K-partite graphs, was used. In these graphs, where relationships are not neglected, variable values are vertices, and disease progression time is captured by topology of value relationships, encoded by edges, connecting the patient's simultaneously observed data value vertices. Importantly, "prototype" data relationship network topologies are completely different in the earliest and latest HCC stages. That gives HCC dynamics monitored by the decrease of influence of early stage prototype-reflected biology and increase of influence of late stage prototype-reflected biology, both independently or in combination. The main discovery of this integrated approach to clinical data processing is that disease progression is not continuous. Instead, HCC patients emerged as naturally grouped into clinically well-separated groups with characteristic ratios of early and late stage biology contributions. We could then start applying the new objective causal stages of HCC to gain new insights into the role of interesting factors, relevant for diagnosis, treatment and prognosis for HCC.

Methods

Clinical Data

4802 HCC patients were identified from our previously published study[7, 8] who had a complete set of values for 17 standard baseline clinical parameters at initial clinical presentation, with tumor size and number and presence or absence of PVT, based on their initial CAT scan measurements and who also had known survival data. The 17 clinical parameters were chosen based upon baseline routine clinical data that is collected to evaluate any newly-presenting HCC patient and are in 3 groups: A), Demographics that included age, gender and HBV/HCV status; B), tumor characteristics, that included maximal diameter (MTD), tumor uni- or multi-focality, portal vein thrombosis (PVT) presence/absence and serum α-fetoprotein (AFP) levels; C), serum liver parameters and blood counts, including levels of albumin, total bilirubin, INR, ALT, AST, ALKP, GGT, Hb and platelets. This work for analysis of de-identified and diseased HCC patients was exempted from written informed consent. ITA.LI.CA database management conforms to Italian legislation on privacy and this study conforms to the ethical guidelines of the Declaration of Helsinki. Approval for the study on de-identified patients was obtained by the Institutional Review Board of participating centers.

Mathematical Methods

NPS determines information about patient time of disease duration at index visit, τ_p from topology of 17-partite graph $\Gamma(\tau_p)$, representing all levels of the time-dependent relationships between all 17 personal coherently observed values (Fig.1). In $\Gamma(\tau_p)$, each diagnostic variable is represented by a

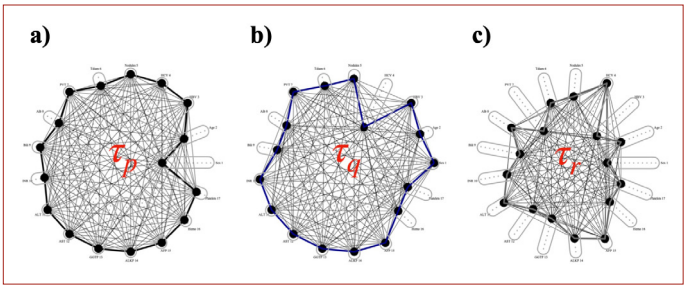

Figure 1. 17-partite graphs $\Gamma(\tau_x)$ for three patients (p, q and r) at different stages of HCC. Ovals - individual clinical variables. Circles in ovals - vertices, representing variable values or intervals of each invariable. Vertices are naturally ordered: values, typically observed at earlier disease stages are on the outside and values observed typically at late stages are on the inside ends of each partition. Solid circles - vertices, representing observed values of respective variables for the patient. Lines - network of relationships between coherently observed values of all variables. Figure demonstrates the change of patient's $\Gamma(\tau_x)$ topology when HCC progresses from early (patient p) through intermediate (patient q) to late (patient r) stages.

partition (ovals in Fig.1). The partition contains the specific set of graph vertices, each representing consecutively ordered intervals of values, covering the entire physiological range of that variable. These intervals are defined by the heterogeneity in the histogram of the variable values, observed in the general HCC population.

The personal network of time-coherently observed data relationships is then represented by the network of edges (lines in Fig.1), connecting the vertices in each partition, representing the observed values of 17 variables. The position of the connected vertex within the complete ordered set of vertices in a variable partition encodes the "first-order", value-based, disease progression information, capturing if the observed value of one variable belongs to "early" or "later" stages of the disease. Edge connections of that variable value to remaining 16 observed variable values, represented by vertices in other partitions, captures the clinical context of the "earliness" or "lateness", seen by the value of the single variable. The "shape/topology" of this network thus directly carries the personalized contextual information about τ_p, defining the disease progression stage.

NPS determines the τ_p from τ_x by computation, derived from 2 fundamental mathematical theorems, one showing that obtaining the best possible τ_p value requires maximization of Fisher information of the data,[9] and another showing that this is achieved by quantifying the differences in topologies between patient $\Gamma(\tau_p)$ and 17-partite prototype graphs.[10] By specific method of finding these prototype topologies, NPS gains the most of new clinical content and explanatory transparency.

First, we examined whether 17-partite prototype networks are different in the earliest and the latest HCC stages. The clinical characteristics of these extreme HCC stages are properties of the disease biology. We therefore needed a network-based clinical disease descriptor. Its construction is straightforward, as we overlayed all individual patient partite graphs into one 17-partite multigraph D. It contains new clinical information through the multiplicities of edges between the variable values: they monitor the clinical relevance of respective observed relationships, quantified by high multiplicities of specific relationship edges in D. This reflects the functional preference for just some combinations of clinical value relationships over all other possibilities, caused by underlying HCC disease biology. We then formulated a "clinically ideal" feature of a networked marker of such HCC biology-determined topology prototype, since its identical copies will be found in D with high abundances. This clinical fact is translated into a "greedy" mathematical procedure by decomposing D into a minimal number of sub-multigraphs with identical multiplicities of all their edges.

An advantage of this approach is that such decomposition is unique[11, 12] for any given disease multigraph D, which can be understood that we obtain "principal topology subgraphs", potentially characterizing disease biology related markers in our data. For our 4802 patients, multigraph D is completely decomposed into 181 principal topology components, representing ~30 reduction in relationship dimensionality.

To find which of these 181 principal topology subgraphs characterize the earliest and the latest HCC stages, we applied the HCC specific adaptation of the general fact that outcome is significantly better for patients in earliest disease stages (HCC patients will be predominantly alive), compared to the outcome for patients in the latest HCC stages (patients will be predominantly dead, 63% in our cohort). We therefore looked for such principal topology prototypes in the set of 181, which, on one hand, share maximum relationship topology features with the most frequent topology features in the sub-group of surviving patients and simultaneously shared a minimal number of such relationships with the topological features, typical for deceased patient subcohort, and vice versa. This identified topology prototypes $P_m'(\tau_{min})$, $P_m''(\tau_{min})$ and $P_x'(\tau_{max})$, $P_x''(\tau_{max})$.

We then used free energy information descriptors[13, 14] to independently quantify content X_p of $P_x(\tau_{max})$ and content Y_p of $P_y(\tau_{min})$ topology prototypes in a patient's observed networked data $\Gamma(\tau_p)$. This results in $[X_p,Y_p]$ NPS characterization of personal HCC stages in a 2-dimensional NPS map (Fig. 2).

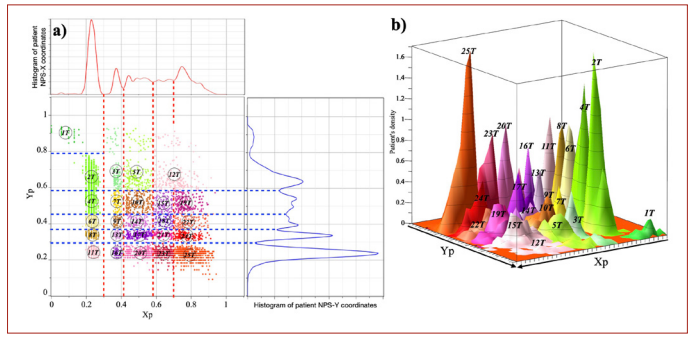

Figure 2. (a) Central panel: NPS 2-dimensional map of disease progression (see text for explanation of clinical meaning of horizontal and vertical axes). Each patient out of 4802 is represented by a point $[X_p, Y_p]$. Right panel - histogram of patient density in the top-down direction of decreasing contribution of early HCC stage biology. Top panel - patient density in the direction of left-right direction of increasing contribution of latest HCC stage biology. Dotted lines - boundaries of HCC progression, separating patient sub-populations in respective NPS phenotype stages sT. **(b)** 3-dimensional histogram of patient population distribution in all 25 HCC NPS phenotype stages sT.

For quantitative analysis and clinical interpretation of trends in the phenotype characteristics, we used the logarithm of abundance of P_y (τ_{min}) in the patient data, which we call (personal) disease burden, DB_p (τ_p). As the X_p and Y_p are expressed in (normalized) logarithmic scale, DB_p (τ_p)=Y_p-X_p. From this definition follows that clinical burden has values between -1 and +1 and is negative for patients with data relationships consistent with dominance of early-stage markers and becomes positive for patients with data relationships showing dominance of late-stage markers. The mathematical details are presented in Appendix 1.

Results

HCC Phenotyping by NPS

We found common networked data relationship "prototype" biomarker patterns P_y (τ_{min}), characteristic for the earliest stages of HCC, and other, completely different, common networked data relationship "prototype" patterns P_x (τ_{max}), characteristic for the latest stages. Different personal times from HCC disease onset are therefore observable by identifying the fractions of the early/late prototype relationship patterns in individual patient actual networked clinical baseline data. This permitted representation of HCC progression in a population as a 2-dimensional disease map, in which a patient is represented in the "HCC clinical progression plane" as a point, with coordinate Y_p, given by the personal content of early-stage HCC prototype topology (vertical axis) and by the content X_p of late-stage HCC prototype topology (horizontal axis), found in the patient's networked data.

We further showed that the distribution of patients in the NPS disease progression map is not uniform. Instead, patients with HCC are in this HCC clinical progression plane map tightly grouped into 25 well-separated sub-cohorts, within which patients are sharing most of their observed clinical feature relationships, with the frequency of their clinical differences decreasing exponentially from the most populated common HCC networked clinical profiles in the subgroup. This allowed us to identify 25 HCC NPS phenotypes sT,s=1…25, which are automatically ordered according to their characteristic disease progression stage (s=1 being the earliest, s=25 being the latest stage), shown in Figure 2.

We then examined the role of currently used clinical decision-supporting factors, in particular of PVT in the full context of other data in the NPS results, identifying and characterizing new clinically relevant categories of PVT patients. For this purpose, we used the fact that NPS analysis resulted in objective (non-statistical) partitioning of the clinically heterogeneous HCC population into stage-specific phenotype sub-populations, sT. These are naturally ordered according to the increasing time from disease onset (or, equivalently, by the increasing disease burden) and, at the same time, patients in sT's are "clinically normalized" to previously unachievable levels. We show evidence that sT's represent non-empirical, most likely disease biology-related stages of HCC.

We computed both phenotype-characteristic disease burdens $DB_s(\tau_s)=\frac{1}{N_s}\sum_{p=1}^{N_s} Y_p - X_p$ as the means of all personal disease burdens for patients from each sT phenotype, as well as the phenotype characteristic mean values $V_s(\tau_s)=\frac{1}{N_s}\sum_{p=1}^{N_s} V_p$ of any clinical variable. Plotting V_s (τ_s)against DB_s (τ_s) provided new clinical insights into HCC by revealing inter-dependencies between the overall disease burden and individual variables, as well as tools to validate that the sT's are indeed ordered by the characteristic increasing time from disease onset.

An example of such dependence, validating this order, is provided by plotting the phenotype-specific mortality as the function of specific disease burden, shown in Figure 3. The line fitted to this trend is a scaled Weibull cumulative distribution function[15] with a shape parameter equal to 2. This function describes the proportionality of deaths in the population to a power of time, providing mathematical confirmation of the theoretically expected functional interdependency.

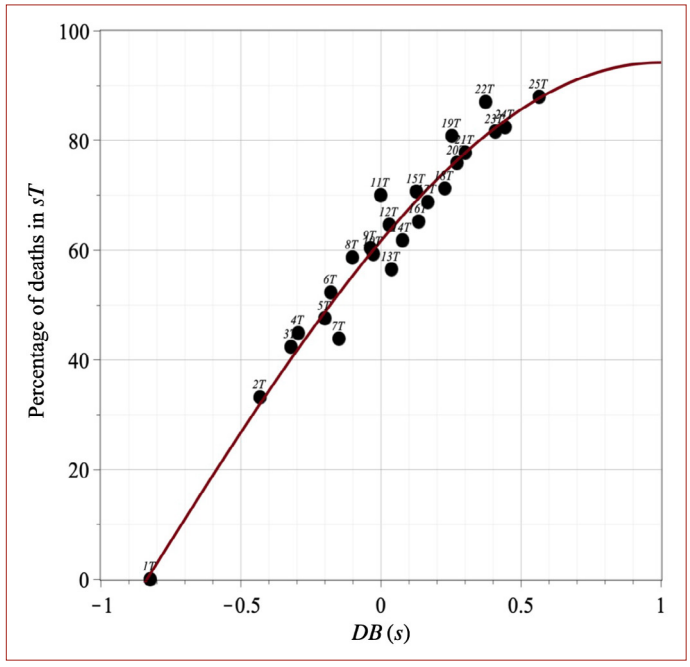

Figure 3. Relationship between the mean disease burden DB[s] and mean mortality for all 25 HCC NPS phenotype sub-populations sT,(s=1…,25)(circles). Least squares fit of the relationship by the Weibull function (lines).

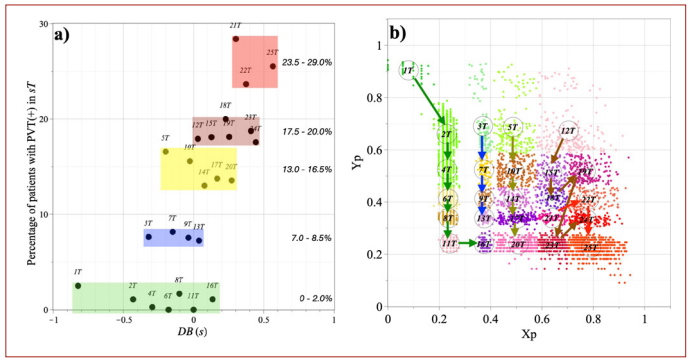

Figure 4. (a) Relationship between the mean disease burden DB[s] and mean PVT incidence for all 25 HCC NPS phenotype sub-populations sT,(s=1…,25). Boxes - patients with specific NPS HCC phenotype stages, constituting the five PVT phenotypes. **(b)** Projection of HCC progression in respective PVT phenotypes. Arrows are colored as the boxes in Fig. 4a.

Practical Example of using the NPS Web-Implementation

For an individual patient, values of all 17 variables recorded within the index visit are collected and entered into the Web tool. First, the diagnosis NPS model computes the compliances [X,Y] of patient's networked input with the early and latest stage biomarkers and shows it graphically by the patient's dot in the www. map. It then uses the result to determine the patient's sT phenotype. Assuming for example that the patient's computed [X,Y] falls into the 12T phenotype region of the map, the web tool shows the percent of deaths for patients in 12T in the training set. These mean mortality values are also shown in Table I: A patient from 12T have ~65% chance of death.

For further personal mortality prognostication, the tool uses 2 pieces of additional clinical information. Firstly, we used follow-up information about time to death for subgroup of training cohort patients in each phenotype, who died within the follow-up interval to determine the mean time to death characteristic of each phenotype. Figure 5 shows that these mean times are inversely linearly related to the sT phenotype-characteristic mean disease burdens: patients who will die with the earliest stage HCC phenotypes have on average ~ 2 times longer time to death than patients in the latest stage HCC phenotypes. HCC stage specific times to death are summarized in Table I. Thus, for our 12 T patient example, the mean time to death is about 34 months.

Secondly, using the www tool, we can further personalize the mortality prediction by using 25 independent NPS dead/alive optimized prognosticating models, one for each sT out of the 25. The input clinical data for 12T patient from our example are automatically re-submitted into the 12th NPS dead/alive prognostication model, resulting in newly computed {xD,yD} coordinates for the patient, which are again shown in second NPS map with only two regions. If the 12T patient NPS result {xD,yD} is in "alive" region, we can expect that he/she is in the smaller ~35% surviving subgroup in this phenotype. By consulting Table I for 12T, the reasonable survival time prognosis for this patient thus will be more than 34 months. For a patient with computed "dead" prognosis, 34 months will be typical time to death. Supplementary Figure 12 shows Kaplan-Meier curves for patients, classified as "alive" or "dead" by the HCC stage-specific NPS mortality prediction models.

PVT Phenotypes

Portal vein tumor thrombus in HCC is a manifestation of aggressive biology and poor prognosis (1-3) and is generally an exclusion criterion for several treatments, such as liver transplantation (4, 16) due to poor outcomes. It is also associated with poor liver function and high tumor recurrence rates after treatment. We therefore analyzed the group within this large cohort of non-surgical HCC patients who had PVT, searching for possible useful prognostic subsets.

First, we determined the characteristic PVT incidence $[PVT]_s = \frac{1}{N_s}\sum_{p=1}^{N_s} V_p(+)$ in every phenotype sT and plotted it against corresponding DB_s (Fig. 4a). It is clear that $[PVT]_s$ is a non-continuous function of disease burden. The 25 primary sT phenotypes form 5 broader groups, shown by differently colored boxes, each with well-separated range of practically progression-independent $[PVT]_s$ percentages.

The explanatory insight into this different behavior of a major negative prognostic factor is provided by projecting the constituent basic phenotypes from every PVT group, shown by colored arrows in Figure 4b. The content of the P_x (τ_{max}) in each patient profile is shown on the horizontal (X) axis. The HCC progression in the respective PVT phenotypes proceeds vertically, top-down, in the respective col-

Table 1. A patient from 12T have ~65% chance of death

	1T	2T	3T	4T	5T	6T	7T	8T	9T	10T	11T	12T	13T	14T	15T	16T	17T	18T	19T	20T	21T	22T	23T	24T	25T
Mean TtD [months]	>60	56	56	53	41	49	43	47	59	47	48	34	45	42	39	43	47	34	33	37	29	33	36	27	28
Mortality [%]	0.0	33.2	42.4	44.9	47.6	52.3	43.9	58.7	60.4	59.3	70.0	64.6	56.5	61.8	70.7	65.2	68.8	71.2	80.8	75.9	77.8	87.0	81.6	82.4	88

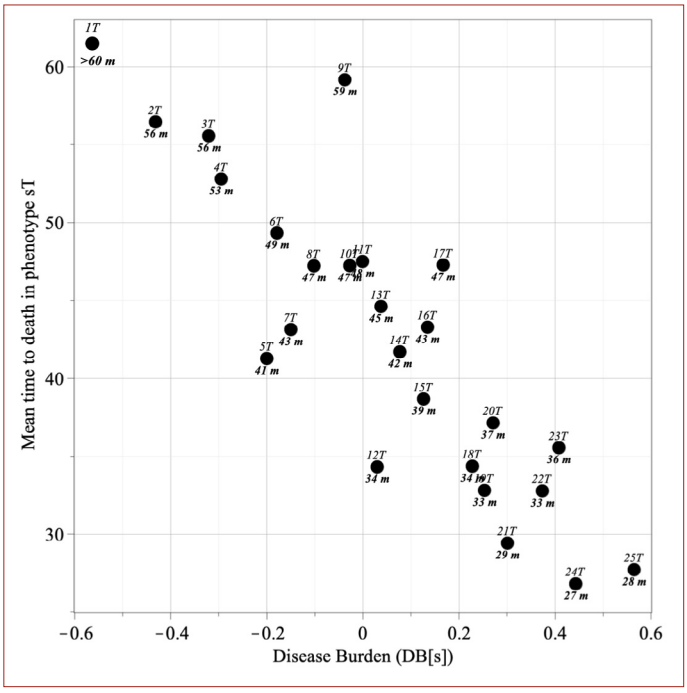

Figure 5. Relationship between the mean disease burden DB[s] and mean time to death (in months) for all 25 HCC NPS phenotype sub-populations sT,(s=1…25). sT = labels above the results, mean time to death = labels below the results. See also Table 1.

umns of the sT fundamental phenotypes (The fluctuations in the last 2% PVT phenotypes are explainable as "noise").

The meaning of vertical top-down progression 1T→2T→4T→6T→8T→11T of the fundamental phenotypes sT in the green PVT group is that, on average, those patients have a small constant content of the "bad" P_x (τ_{max}) relationship markers (x-coordinate is not changing in the progression). This shows that gradual disappearance of the "good" P_y (τ_{min}) relationship features as HCC goes from top (early) to down (later) stages exclusively monitors the HCC progression in this sequence of sT's.

Thus, the patients from green PVT cohort present with minimal and constant evidence of the "bad" HCC biology, related to the latest HCC stages. The patients from blue PVT subgroup present with a larger constant content of the "bad" P_x (τ_{max}) than the green PVT subgroup. Thus, the biological heterogeneity of blue PVT patient subgroup is higher than that of the green PVT patient subgroup. The yellow PVT patient subgroup contains even larger constant evidence of their "bad" biology than the blue PVT subgroup (and of course much higher than green PVT subgroup). The same holds (with some noise, which is relatable to the dominancy of P_x (τ_{max}) and minimal content of P_y (τ_{min}) for brown and red PVT subgroups.

In summary, each PVT subgroup has a constant, characteristic increase in content of P_x (τ_{max}), which defines the constant frequencies of occurrence of PVT in them. The P_x (τ_{max}) contains the relationships between values of 17 variables, which are characteristic for late stages of HCC. This is responsible for the increase of PVT pincidence in the sequence of the 5 PVT groups, progressing from green to red. This is an example of an important function of NPS, because its results are not statistical, but derived from functional clinical first principles, they are hypothesis-generating, directly providing information for designing validation trials. For using this result in our practical example, we identify the patient 12T phenotype in Figure 4a, assigning him to "brown" group with 18-20% incidence of PVT.

Discussion

The novelty of NPS methodology in clinical analytics originates from integrating all the patient- and time-coherently observed relationships between multiple clinical variable values into K-partite graph-based processed input. Simultaneously, new clinical content in NPS results becomes available since we integrated the appropriate tools of discrete mathematics, general physics and theoretical informatics to obtain the implementable and completely scalable functional, non-statistical mathematical processing of this "new" information in "old clinical data", without encountering the "complexity catastrophe" that would be encountered by conventional methodologies.

Another novel feature of NPS approach is change of its founding clinical principle from conventional frequentist's paradigm of average impact of disease on any patient to determining the personal time of disease duration τ_p at the index clinical visit when the data are collected. This enables explicit characterization of disease as a dynamic process using apparently "static" clinical data. Here we show by characterizing the HCC progression that networked relationships between coherently observed values of multiple informative clinical variables encode enough information about the personal disease progression history for NPS to extract τ_p from their topology.

This is mainly due to the existence of networked data relationship "prototype" biomarker patterns P_y (τ_{min}), characteristic for the earliest HCC stages, and completely different, common networked data relationship "prototype" biomarker patterns P_x (τ_{max}), characteristic for the latest HCC stages. Different personal times τ_p from HCC disease onset are therefore observable by identifying the contributions of the early/late topology prototype relationship biomarker patterns to the patient's actual networked clinical baseline data. This result allows the representation of the HCC dynamics as a 2-dimensional disease progression map, in which a patient is represented as a point [X_p, Y_p],

with coordinates, given by the personal content of early-stage HCC prototype topology (vertical axis) and content of late-stage HCC prototype topology (horizontal axis), found in the patient's integrated and networked data.

The results of these NPS data processing steps represent discoveries of new clinical features, providing new insights into HCC biology or allowing formulation of new testable hypotheses about HCC biology. We identified 25 objectively defined HCC phenotypes sT, each with clearly distinguishable clinical characteristics, which determine the τ_s-defined order of these stages (from s=1 to s=25). The main discovery of NPS is that the disease burden, imposed upon the patient by HCC stage sT, is not continuous, but proceeds by stepwise transitions between "clinically stable" disease stages.

Applicability of this NPS result in diagnostic clinical practice stems from the fact, that there are significantly large sub-populations of HCC patients in each sT HCC stage phenotype.

Applicability of this NPS result in prognostic clinical practice stems from disease progression-defined ordering of the NPS phenotypes. If we determine that patient is in HCC stage sT at baseline, then the NPS phenotype ordering indicates that the disease progression for that specific patient will be either stable disease or progression to (s+1)T stage or (possibly treatment-related) improvement to (s-1)T.

This set of τ_s-ordered HCC phenotype subsets also provide detailed insight into HCC biology by allowing us to examine changes in the observable functional background and the presence of established risk and treatment-decision factors as a function of disease progression. We used the example of presence of PVT, a major negative prognostic factor for HCC. Therefore, clinical predictive factors for presence of PVT and identification of patient subgroups having PVT with differing survivals is a crucial part of HCC patient management. We hypothesize that this might be biologically related to the reported stem cell marker heterogeneity, which is open to experimental validation. So far, the main identified HCC patient risk factors for PVT development include large (>5cm) HCC size, elevated AFP levels (>500 IU/mL), low serum albumin levels and male gender, while predominant prognostic factors include serum AFP and albumin levels and indices of inflammation.[17-19]

Our NPS may also help explain different survival outcomes for patients having the same treatment. This may be a reflection of known tumor heterogeneity and evolution that has derived from multiple studies of histopathology, tumor mutation burden and satellite instability. We know that tumors are not static in their composition. Our findings reflect that. There are likely multiple pathways to tumor growth, as well as changes in the pathways during various phases of growth of a tumor in an individual patient. Our τ_s-ordered stages reflect quantitatively the clinical observations of these events. It is also true than in most series, the percent of patients with PVT increases with MTD up to about 50%, but there are still about 30% of patients with large MTD >8cm without PVT.[20] Perhaps, the same stem cell that causes increasing MTD might also (with input from other factors) cause an increase in PVT. Or perhaps a patient's HCC has different stem cells.

Conclusion

NPS uses a personal networked relationship-based characterization of the patient clinical status, which is never partitioned (step 1). From these personal networks, NPS constructs the characterization of HCC disease, allowing a determination of prototype-networked biomarkers for earliest and latest HCC stages (step 2). These 2 steps result in the possibility of characterizing the personal disease progression stage at baseline, by determining compliance of patient actual clinical data network with early-stage prototype networked biomarker (compliance descriptor Y) and with final stage prototype networked biomarker (compliance descriptor X). Patient HCC progression is then defined by the [X, Y] stage point in the 2D-HCC progression map (step 3). This map directly shows that HCC disease appears to be stable in 25 clinically relevantly large sub-populations of patients 1T… 25T. The stable HCC disease of a patient in stage sT progresses to different stage rT only after the accumulation of a critical amount of new personal relationships, which will change the 2 personal compliances [X, Y] of the personal networked profile to those in another phenotype rT, which is different from sT. Further improvement of clinical insight into HCC biology can be obtained in future by integrating additional coherently acquired patient data with our 17 by the NPS approach, which can seamlessly integrate new types of clinical data.

Appendix: *https://jag.journalagent.com/jilti/abs_files/JILTI-63935/JILTI-63935_(1)_JILTI-63935_(0)_Appendix_(son).pdf*

Disclosures

Ethics Committee Approval: This work complies with the guidelines of the World Medical Association, Declaration of Helsinki. This work was approved by our institution's IRB as documented in the methods section

Peer-review: Externally peer-reviewed.

Conflict of Interest: The authors declare no conflict of interest. All authors have read and agree with the contents of this paper.

Strobe Statement: The authors have read the STROBE statement – checklist of items, and the manuscript was prepared according to its checklist of items.

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Original Research

Salvage Living Donor Liver Transplantation for Best Supportive Care Patients with Advanced HCC

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Abstract

Objectives: Patients with advanced stage hepatocellular carcinoma (HCC) and liver decompensation have been suggested to receive best supportive care (BSC) according to BCLC algorithm and these patients have a median estimated survival of only 3 months (1). By contrast, living donor liver transplantation (LDLT) performed in a subgroup of BSC patients may not cure the advanced cancer, but it can cure the liver dysfunction. Thus, even if the tumor recurs after transplantation, patients can be treated with local or systemic therapies due to their good liver function, with potential for longer survival. The aim of this study was to compare the survival of BSC patients versus salvage LDLT (sLDLT).

Methods: The data of 492 LT patients with HCC were analyzed retrospectively from our databank, which is recorded prospectively and sequentially (2). Among these LDLT patients, those with Child class C and advanced stage HCC [beyond Expanded Malatya criteria] (3) without extrahepatic metastasis aged between 18-60 years were included in the study as the sLDLT group. The data of non-transplant HCC patients were also reviewed and BSC patients were included as BSC group. The survival of sLDLT and BSC groups was then compared.

Results: sLDLT group had 17 patients and BSC group had 48 patients. Median survivals were 1020 days (291.6 – 1748.4, 95% CI) in sLDLT group and 40 days (30.9 – 49.1, 95% CI) in BSC group. Hospital mortality (<90 days) in sLDLT group was 2 patients (11.7%), and in BSC group was 81.3% (39/48). Post-LDLT recurrence rate was 66.7% (10/15) and 3-year overall survival (OS) was 50%. We then dichotomized the LDLT group into >2 years and <2 years survival, patients who survive >2 years had significantly lower MTD (2.5 vs 7.5 cm, p=0.036) and lower platelet levels (60.5 vs 93, p=0.027)

Conclusion: No palliative treatment could result in 50% 3-year OS in the BSC patients. However, we could achieve 3-year OS of 50% in selected patients in the BSC group (No extrahepatic metastasis, Child C and ages between 18-60) by LDLT.

Keywords: BSC, Palliative, LDLT, live donor, macrovascular invasion

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Liver transplantation (LT) is a potentially curative treatment for HCC, while palliative treatments are not curative. These 2 terms should not be used together in a single sentence. However, as surgeons, we operate on tumor patients for 2 main goals. The first goal is to cure the cancer which means long term and tumor free survival, and the

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second goal is to ensure that patients have some quality of life for the rest of their lives. Based on the second goal, the question arose as to whether we can use living donor liver transplantation (LDLT) as a palliative treatment in patients with advanced hepatocellular carcinoma (HCC).

Patients with advanced stage HCC and liver decompensation have been suggested to only be eligible for best supportive care (BSC) according to the BCLC algorithm and these patients have only a median 3 month estimated survival.[1] On the other hand, LDLT in a subgroup of BSC patients, may not cure advanced cancer, but it can normalize the liver function. Thus, even if the tumor recurs after transplantation, patients can be treated with local or systemic therapies due to good liver function post-transplant with potential for a longer life.

The aim of this study was to compare the survival of BSC patients with salvage LDLT (sLDLT).

Methods

Ethics

This study has been approved by Inonu University Ethics Committee with approval no: 2024/6410. Due to this study having a retrospective design, informed consent from patients was not necessary.

Study Population

Between March 2002 and July 2024, 592 LTs were performed at the Liver Transplantation Institute of Inonu University for patients with HCC.[2] We retrospectively analyzed the data of LT patients with HCC from the database which is recorded prospectively and sequentially.

Patients beyond Expanded Malatya Criteria[3] were reviewed and the patients who met the inclusion criteria were analyzed.

Inclusion Criteria

1. Child-Pugh class C.
2. HCC limited in the liver (no extrahepatic spread).
3. Age between 18 and 60.

Data of BSC patients were reviewed from the whole HCC council databank of the Liver Transplantation Institute of Inonu University.

Patient demographics, tumor morphology (according to explant pathology report), etiology of the underlying liver disease, pre-transplant laboratory values, Child-Pugh class, MELD Na score, graft to recipient weight ratio (GRWR), overall survival (OS) years and post-transplant recurrence rate were recorded.

Surgical Technique and Management of Immunosuppressive Treatment

Our patient selection criteria, surgical method in LT for HCC, and immunosuppressive treatment protocol have been described in our previous studies.[4-8]

Statistical Analysis

Normality of the quantitative data was assessed by Shapiro-Wilk test and summarized by median and interquartile range (IQR). Mann-Whitney U test was used to compare two independent groups. The distribution of the qualitative data was presented by count and percentage. Exact chi-square tests were used for comparisons according to categorical data. The two-sided significance level was considered as 0.05 in all analysis.

Survival analyses were performed using the Kaplan-Meier method, Log-Rank test, and Cox regression analysis. The two-tailed significance level was set at 0.05. Overall survival defined as the time between the transplant day and death and calculated as years and death patients were censored by Kaplan-Meier method. All statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp, Armonk, NY).

Results

There were 17 patients who met the inclusion criteria in the sLDLT group, and 48 in the BSC group. Patient demographics were summarized in Table 1.

Patient Demographics

sLDLT and BSC groups were statistically different in terms of age, MELD score, liver function tests, and AFP levels but were similar in terms of BMI, BSA, MTD, number of nodules, etiology and gender. The 62.5% (30/48) of the BSC patients were metastatic.

The median age, MELD Na score, AFP, MTD and number of nodules in sLDLT group were 53 years (41 – 59), 21 (16-34), 184ng/ml (2.0-14560), 4.5 cm (2.0-20), 4 (1-11), respectively.

The median age, MELD Na score, AFP, MTD and number of nodules in BSC group were 64 years (37 – 84), 14 (6 – 25), 1000 (1.67 – 97248), 11 cm (1.8 – 25), 2 (2-11), respectively.

In the sLDLT group, 82.4% of patients were male and the major etiology was HBV cirrhosis in 58.8%, while in the BSC group, male gender was 91.7% and HBV was 35.4%. Patient demographics were summarized at Table 1.

Patient Mortality Rates

Two patients in the sLDLT group died in the early post-LDLT period due to sepsis. Hospital mortality rate (<90 days) in the sLDLT was 11.7% (2/17).

Table 1. Demographics of the patients

Parameters	pLDLT (n=17) Median (min-max)	BSC (n=48) Median (min-max)	p
Age	53 (41 – 59)	64 (37 – 84)	<0.001
MELD score	21 (16 – 34)	14 (6 – 25)	<0.001
BMI	24.7 (19.1 – 35.6)	24.0 (17 – 50)	0.727
BSA	1.96 (1.55 – 2.25)	2.00 (1.53 – 2.82)	0.523
GRWR (%)	1.06 (0.77 – 1.87)		
Albumin	2.4 (1.2 – 3.6)	3 (1.7 – 3.9)	0.012
Total Bilirubin	5.15 (2.1 – 16.7)	3 (0.5 – 27.1)	0.049
INR	1.52 (1.25 – 3.19)	1 (0.9 – 1.8)	<0.001
Platelet	84 (23 – 197)	169 (11.2 – 405)	<0.001
AST	176 (57 – 7789)	134 (38 – 397)	0.402
ALT	98,5 (38 – 3535)	55 (24 – 226)	0.001
GGT	100.5 (29 – 192)	234 (37 – 1179)	<0.001
ALP	148 (28 – 2327)	231 (74 – 1649)	0.003
AFP	184 (2.0 – 14560)	1000 (1.67 – 97248)	0.018
WBC	4.5 (1.8 – 29.3)	8 (2.5 – 28.6)	0.014
MTD (cm)	4.5 (2.0 – 20.0)	11 (1.8 – 25)	0.126
Number of nodules	4 (1 – 11)	2 (2 -11)	0.766
NLR	6.66 (1.62 – 35.3)	4.24 (0.94 – 16.6)	0.510
PLR	110 (38.05 – 252.5)	161.5 (16 – 876.6)	0.130
CRP	2.35 (0.3 – 41.7)	3 (0.38 – 17.1)	0.522
	n (%)	**n (%)**	**p**
Gender			
Female	3 (17.6)	4 (8.3)	0.287
Male	14 (82.4)	44 (91.7)	
Differentiation			
Well	1 (5.9)		
Moderate	8 (47.1)		
Poor	8 (47.1)		
Venous invasion			
None	2 (11.8)		
Micro (+)	8 (47.1)		
Macro (+)	7 (41.2)		
Recurrence			
Yes	10 (58.8)		
No	7 (41.2)		
Extrahepatic disease			
Yes	0 (0)	30 (62.5)	<0.001
No	17 (100)	18 (37.5)	
Etiology			
Cryptogenic	4 (23.5)	19 (39.6)	0.129
HBV	10 (58.8)	17 (35.4)	
HBV+HDV	2 (11.8)	1 (2.1)	
HCC	1 (5.9)	10 (20.8)	
HCV	0 (0)	1 (2.1)	
GRWR, %			
≥0.8	16 (94.1)		
<0.8	1 (5.9)		
TTD, cm			
≤8	6 (35.3)		
>8	11 (64.7)		

All patients died in the BSC group before 1 year. Mortality rate within 90 days in the BSC group was 81.3% (39/48).

Patient Overall Survival

Median overall survival (OS) for the sLDLT group (n=17) was 1020 days (almost 3 years) (62.0 – 1977.9 days, 95% CI), and for the BSC group was 40 days (30.9 – 49.1 days, 95% CI) p<0.001 (Fig. 1).

3-year OS rate was 50% and post-Tx recurrence rate was 66.7% (10/15) in the sLDLT group.

Post-Transplant Recurrence

Post-transplant recurrence was detected in 10 patients in the sLDLT group. One patient had only hepatic recurrence which is unresectable treated by systemic chemotherapy, 4 patients had only extrahepatic disease (1 bilateral lungs treated by systemic chemotherapy, 2 had vertebra treated by Radiotherapy and Cyber knife, 1 brain metastases treated by surgical resection), and 5 patients had both hepatic and extrahepatic disease treated by systemic chemotherapy. In Türkiye, immune check point inhibitors are not covered by the government so, only tyrosine kinase inhibitors (sorafenib as first line) are reimbursement by Ministry of Health. Only the patient who had brain metastasis treated by surgical resection is still alive since 1714 days from transplantation (4.7 years), 653 days from the recurrence (1.8 years), remaining all recurrent patients were died.

Subgroup Analysis of the sLDLT Patients

The LDLT group was subsequently dichotomized into >2 years and <2 years survival groups, patients who survive >2 years had significantly lower MTD (2.5 vs 7.5 cm, p=0.036) and lower platelet levels (60.5 vs 93, p=0.027) (Table 3).

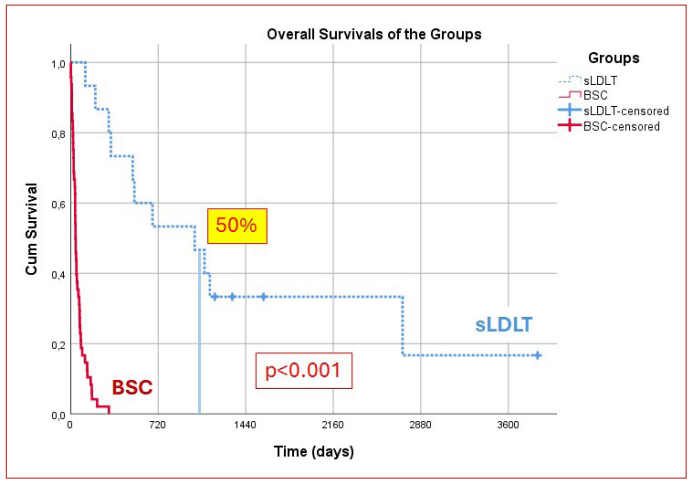

Figure 1. Overall survivals of the groups.

Table 2. Comparison of treatment modalities for advanced HCC

Study, year	BSC	ICI	TKI	TACE	LDLT	p
Xia J, et al, 2021						
Median survival, months	1.3	3.3	3.1			<0.05
Fulgenzi CAM, et al, 2024						
Median survival, months	4.04	7.5				<0.001
Akarapatima K, et al, 2022						
Median survival, months	8.2			21.4		<0.001
Xiang X,et al, 2019						
Median survival, months	6			9		0.007
Llovet JM, et al, 2008						
Median survival, months	7.9		10.7			<0.05
Yau T, et al, 2019						
Median survival, months		16.4	14.7			0.752
Cheng AL, et al, 2022						
Median survival, months		19.2	13.4			<0.001
Malatya experience, 2024*						
Median survival, months	1.3				36	<0.001

BSC: Best supportive care; LDLT: living donor liver transplantation; ICI: Immun-check point inhibitors; TKI: tirosin kinaz inhibitors; TACE: Transarterial chemoembolization; *: current study.

Discussion

Best supportive care has been suggested for HCC patients with terminal stage (D) disease, according to the updated Barcelona Clinic Liver Cancer Staging System in 2022 with an estimated survival of only 3 months for these patients.[1] The BCLC Stage D is defined as patients with any tumor burden and end stage liver function and ECOG performance score 3-4.[1] We have a weekly liver tumor board in our center and we discuss all tumor patients in a multidisciplinary manner.[9] HCC patients beyond the Expanded Malatya criteria with macrovascular invasion and age >70 years with Child class C cirrhosis and/or extrahepatic spread are normally suggested to be offered only BSC by our tumor board. The 62.5% of the BSC patients had extrahepatic disease and the rest of them had macrovascular portal vein and/or hepatic vein tumor thrombosis with decompensated liver functions.

As a result of the accumulated LDLT experience in our center, we have seen that the LDLT outcomes of some patients who were suggested for BSC were encouraging. So, we reviewed our transplant-HCC databank and generated the sLDLT group. LDLT patients with Child class C and advanced stage HCC (beyond Expanded Malatya criteria) without extrahepatic metastasis and ages between 18-60 years were included into the sLDLT group. The data of non-transplant HCC patients were also reviewed and BSC patients were included as the BSC group. We found remarkable survival differences between the groups. The median survival time in the BSC group was 40 days, while the 3-year overall survival rate in the sLDLT group was 50%. However, the recurrence rate after Tx was very high at 66.7% as expected, but these patients were able to receive local or systemic treatments due to their good liver function resulting from their liver transplantation.

Can LDLT be Used as a Palliative Treatment in Selected Patients within the BSC Group?

LDLT has some advantages and disadvantages. In the LDLT procedure, the graft is a personal gift from the recipient's family, so there is no harm to those on a transplant wait list. Quality of the graft used in LDLT and LDLT gives a chance of saving or improving the life of their beloved recipients for the donors. Centers in Türkiye are very experienced on LDLT. On the other hand, LDLT has some disadvantages. There is donor risk in LDLT procedure and risks a life to save another life. What should be the minimum recipient survival that would be worth risking the donor? According to the transplant community, minimum expected (acceptable) recipient survival to cover these risks should be >50% at 5 years.[10, 11] In a study on donor candidates from Canada, donor candidates agree to be a donor if the recipient's life expectancy after LDLT will be extended by at least 11±22 months on average.[12] Basic ethical principles of LDLT are autonomy, altruism, utilitarianism, beneficence, non-maleficence and justice. The basis of live donation is a selfless gift to others, without donor coercion, voluntarily, without any payment, and solidarity between donor and recipient. To prevent coercion, we established a "plausible deniability" mechanism. Donors have the chance to opt out of LDLT confidentially at four stages during screening, making them ineligible if they choose to opt out. When a donor is declared ineligible for LDLT/LPE, whether due to opting out or any other reason, only the donor is informed about the cause of their ineligibility. Written informed consent for procedures and anesthesia is obtained from each individual.[13] In light of these ethical principles, they donate in order to benefit their loved ones. Therefore, minimum donor risk and maximum recipient benefit must be targeted.

According to our results, donor complications are acceptable[14, 15] and sLDLT patients have a 3-year overall survival of 50%. In addition, hospital mortality (<90 days) in sLDLT was 11.7%, while in BSC it was 81.3%. Based on these results, LDLT can be considered as a palliative method for selected patients in the BSC group.

There are numerous studies comparing treatment modalities with BSC in terms of survival for advanced HCC, as shown in Table 2.[16-22] In fact, these comparisons are not appropriate, because on the one side there are BSC patients who cannot receive any treatment due to impaired liver function, and on the other side there are patients with

Table 3. Subgroup analysis of the pLDLT group (n=17)

	Survival≤2 years (n=9)		Survival >2 years (n=8)		
	n	**Median (IQR)**	**n**	**Median (IQR)**	**p**
Age at Tx date	9	54 (8.5)	8	50.5 (9.5)	0.423
MELD score	9	24 (11)	8	20 (6.75)	0.743
BMI	9	25.7 (4.59)	8	23.81 (4.25)	0.606
BSA	9	1.97 (0.27)	8	1.86 (0.4)	0.370
GRWR (%)	9	1.04 (0.55)	8	1.16 (0.39)	0.673
Albumin	8	2.4 (1.17)	8	2.4 (0.93)	0.878
Total Bilirubin	8	5.67 (7.1)	8	4.95 (6.27)	0.798
INR	8	1.52 (0.44)	7	1.56 (0.7)	0.955
Platelet	9	93 (71.5)	8	60.5 (53.25)	0.027*
AST	8	210.5 (172)	7	144 (191)	0.397
ALT	8	122.5 (152)	8	83 (83.75)	0.279
GGT	8	141 (115)	8	56 (96)	0.234
ALP	8	127 (106)	8	158.5 (89.25)	0.195
AFP	9	227.9 (5408.95)	8	26.7 (416.47)	0.093
WBC	9	6.72 (8.25)	8	4.39 (1.06)	0.277
RDW	8	16.8 (4.92)	8	17.3 (4.95)	0.959
MTD (cm)	9	7.5 (5.25)	8	2.5 (2.5)	0.036*
Number of nodules	9	3 (9)	8	4 (8)	1.000
NLR	8	5 (4.68)	8	7.37 (9.33)	0.574
PLR	8	99.1 (79.61)	8	124.17 (159.74)	0.959
MPV	8	10.9 (2.02)	8	10.1 (3.53)	0.645
CRP	4	5.34 (30.64)	6	2.22 (4.54)	0.171
	n	**(%)**	**n**	**(%)**	**p**
Gender					
Female	1	(11.1)	2	(25)	0.576
Male	8	(88.9)	6	(75)	
Differentiation					
Well	0	(0)	1	(12.5)	0.798
Moderate	5	(55.6)	3	(37.5)	
Poor	4	(44.4)	4	(50)	
Venous invasion					
None	0	(0)	2	(25)	0.440
Micro (+)	5	(55.6)	3	(37.5)	
Macro (+)	4	(44.4)	3	(37.5)	
Recurrence					
Yes	3	(33.3)	4	(50)	0.637
No	6	(66.7)	4	(50)	
AFP, ng/ml					
≤200	3	(33.3)	6	(75)	0.153
>200	6	(66.7)	2	(25)	
GGT, IU/L					
≤104	3	(37.5)	5	(62.5)	0.619
>104	5	(62.5)	3	(37.5)	
Etiology					
Cryptogenic	2	(22.2)	2	(25)	1.000
HBV	5	(55.6)	5	(62.5)	
HBV+HDV	1	(11.1)	1	(12.5)	
HCC	1	(11.1)	0	(0)	
GRWR, %					
≥0.8	8	(88.9)	8	(100)	1.000
<0.8	1	(11.1)	0	(0)	
TTD, cm					
≤8	2	(22.2)	4	(50)	0.335
>8	7	(77.8)	4	(50)	

preserved liver function who can receive local or systemic treatments. Consequently, patients who can receive any palliative treatment live longer than BSC patients, but no palliative treatment can result in 50% 3-year survival, except sLDLT.

Post-transplant recurrence rate were high in the sLDLT as 66.7%. Fifty percent of recurrences were both hepatic and extrahepatic disease, 40% (4/10) of recurrence were only extrahepatic disease and local treatments such as surgical resection, radiotherapy were used for this patients. All recurrent patients who had systemic disease or can not treat locally had sorafenib as a systemic chemotherapy. One patient whose brain metastasis treated by surgical resection is still alive for 1714 days from the LDLT, 653 days from the recurrence time. Immune check point inhibitors are not reimbursement yet in Türkiye, but in 2025 it will be covered by the social insurance. Oncologic treatments are developing quickly, so future treatments can offer better survival for these patients, and sLDLT can add extra years for selected BSC patients.

We divided the sLDLT group into 2 subgroups, namely those who survived more than 2 years and those who survived less than 2 years and we then examined the characteristics of those who survived longer in terms of tumor and laboratory parameters. Patients who survive >2 years had significantly lower MTD (2.5 vs 7.5 cm, p=0.036) and lower platelet levels (60.5 vs 93, p=0.027) (Table 3). This finding can help explain the survival difference.

Limitations of this study are the retrospective design and the small patient numbers, absence of quality-of-life data but the strength of this study is that it is the first report on this subject, which is promising.

Conclusion

We could achieve 3-year OS 50% in selected patients from the BSC group (no extrahepatic metastasis, Child C cirrhosis and ages between 18-60) by LDLT, although the post-Tx recurrence rate was 66.7%. No palliative treatment could achieve 50% 3-years overall survival in BSC patients other than LDLT.

Disclosures

Ethics Committee Approval: This study has been approved by Inonu University Institutional Review Board (Approval no: 2024/6410).

Peer-review: Externally peer-reviewed.

Data Availability: The raw data used to support the findings of this study are available from the corresponding author upon request.

Informed Consent: No informed consent was requested from patients since this is a retrospectively designed study.

Author Contributions: Concept – V.I., F.O.; Design – V.I., F.O., S.U., B.I.C., S.Y.; Supervision – B.I.C., B.I., S.Y.; Funding – None; Materials –None; Data Collection and/or Processing – V.I., F.O., S.U.; Analysis and/or Interpretation – H.G.B, V.I., S.U.; Literature Review – V.I., F.O., B.I.C.; Writing – V.I., F.O.; Critical Review –B.I.C., B.I., S.Y.

Declaration of Interests: The authors have no conflict of interest to declare.

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Original Research

The Efficacy of Apigenin in the Treatment of High-Grade Hepatocellular Carcinoma: An Invitro Experiment

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Abstract

Objectives: Apigenin, a flavonoid with reported antineoplastic and anti-inflammatory properties, is being investigated for its potential in treating hepatocellular carcinoma (HCC). This study evaluated apigenin's effects on proliferation, invasion, and viability of the SNU-449 HCC cell line.

Methods: To evaluate apigenin's antiproliferative and antimetastatic effects in HCC, we performed MTT assays at 24, 48, and 72 hours, using six apigenin concentrations (2.5–100 µM). Following the determination of the minimum effective concentration at 48 hours, SRB, colony formation, and wound healing assays were performed at that dose. All results are expressed as median (interquartile range).

Results: The MTT assay identified 5 µM apigenin at 72 hours as the minimum effective dose. Absorbance at 5 µM apigenin and in the untreated control was 0.581 (IQR: 0.26) and 0.67 (IQR: 0.049), respectively (p>0.05). The SRB assay showed no significant difference between the apigenin-treated and control groups (0.54 [IQR: 0.07] vs. 0.381 [IQR: 0.365]; p>0.05). The colony formation assay revealed a modest reduction in survival fraction in the apigenin-treated group (74% relative to control). Wound areas at the end of the wound healing assay were 528,366 (IQR: 691,200) µm^2 in the apigenin-treated group and 528,861 (IQR: 523,150) µm^2 in the control group (p>0.05). Wound closure rates were similar between the apigenin-treated and control groups (59.5 [IQR: 36.9]% vs. 59.75 [IQR: 15.4]%; p>0.05).

Conclusion: The results of this study suggest that apigenin's direct antiproliferative and antimetastatic effects on HCC cells may be limited. Further research focusing on the modulation of the tumor microenvironment and the induction of antitumor immune responses could provide valuable insights.

Keywords: Apigenin, Antimetastatic treatment, Antiproliferative effects, Hepatocellular cancer, SNU-449 cell line

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Hepatocellular carcinoma (HCC) is the most frequently observed major liver neoplasm type and is predominantly accountable for liver cancer. It is becoming increasingly widespread worldwide and it displays poor prognosis.[1] Throughout the spectrum of health problems, liver cancer is the second major source of cancer-associated death.[1] Even though advanced technology and therapeutic forms have resulted in a substantial abridgment of the death rate caused by main cancer kinds, advancement in therapies for liver cancer has stayed mostly stagnant. Therefore, the development of better liver cancer therapies is deeply needed.[2]

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Apigenin is a naturally occurring polyphenol of the flavonoid family, abundant in everyday food items such as vegetables, fruits, and tea. There are emerging reports indicating that apigenin applies anti-inflammatory, anti-tumor, and pro-apoptotic properties.[3] Recent research indicates that apigenin notably reduced the development, multiplication, and expansion of liver cancer cells in treated rats.[3,4] Petroleum ether-extracted propolis elements highly enriched in apigenin significantly repressed the advancement of liver cancer in mice; this could transpire via adjusting cell cycle and prompting cell apoptosis.[3,4] Redox alteration is a main component in the tumorigenesis of hepatocellular carcinoma (HCC) in individuals and animal models. Studies have shown that an apigenin-rich diet substantially promptly ameliorated oxidative stress and decreased HCC expansion in rats.[5,6] Additionally, a variety of research reported that apigenin suppressed liver cancer advancement by prompting a rise in oxidative stress in numerous cell lines and animal models. Bioinformatical and experimental analyses have demonstrated that upregulated miR-520e prompted by apigenin holds back coding mRNA by binding to the 3'-UTR, prompting a rise in ROS, ultimately resulting in tumor cell apoptosis.[5-8]

Apigenin (4',5,7-trihydroxyflavone) is a natural compound that can be found in high levels in parsley, onions, oranges, tea, chamomile, cereal, fruit, and some herbs.[9] As a natural source of food and ingredient in traditional Chinese medicine, there are some reports pointing to low toxicity and high efficacy in the prevention and treatment of cancers.[10-12] Recent research demonstrated that apigenin could significantly regulate the PI3K/Akt signaling pathway and ultimately suppress the proliferation of hepatocellular treatment by halting the cell cycle and causing apoptosis, thus showing the potential of apigenin to act as a novel drug in the treatment of HCC.[9] In another recent vivo experiment, the results indicated that apigenin potentially inhibits the focal damage of liver tissue. Chronic CCl4-exposed rats supplemented with apigenin diminished lipid peroxidation, restored the defenses of superoxide dismutase and glutathione peroxidase, boosted the level of catalase, and reduced alanine transaminase, aspartate aminotransferase, and γ-glutamyl transpeptidase in serum. Furthermore, apigenin enhances the apoptosis of liver cells and curbs the chemical changes of the liver microenvironment induced by CCl4, therefore exerting a therapeutic effect on hepatocirrhosis.[13-15] More notably, accumulating evidence demonstrates the superior bioavailability of apigenin among flavonoids. These findings collectively underscore apigenin's potential as a safe and practical drug. Due to the pleiotropic properties of apigenin in viruses, for example, anti-proliferate, anti-angiogenic, antioxidative, and anti-proinflammatory actions, as well as its inhibitory effects on mitochondrial biogenesis, there is an urgent need for relevant trials of exploration targeting apigenin supplementation to develop drug therapy for liver disorders and HCC.[15-17]

SNU449 cell line (ATCC® CRL-2234™) is an HCC cell line with grade II/III differentiation that developed on the background of hepatitis B virus (HBV) related cirrhosis.[18] The cell line is positive for HBV deoxyribonucleic acid (DNA). It can be used in experiments that require biological characteristics and behavior of HCC.[18] We designed the study aiming to evaluate the invitro biological effects of apigenin on the proliferation and migration of SNU449 HCC cell lines.

Methods

Cell Culture

Human hepatocellular carcinoma SNU-449 cells (ATCC, CRL-2234) were maintained in RPMI-1640 medium (Sigma) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Sigma) and 1% penicillin-streptomycin-neomycin (Sigma) under standard cell culture conditions (37 °C, 5% CO_2, humidified atmosphere).

Cell Viability Assay

SNU-449 cells were resuspended in RPMI-1640 medium and seeded at a density of 10,000 cells per well in 96-well plates. Cells were treated with apigenin (Sigma) at concentrations of 2.5, 5, 10, 20, 50, and 100 µM in RPMI-1640 for 24, 48, and 72 hours. Following the designated incubation period, MTT solution (5 mg/mL in PBS) was added to each well and incubated for four hours. Subsequently, 100 µL of dimethyl sulfoxide (DMSO, Merck) was added to dissolve the formed formazan crystals. Absorbance was measured at 570 nm using a microplate reader (Biotek, Synergy H1m), and the IC50 value was determined.

Sulforhodamine B (SRB) Assay

SNU-449 cells were seeded at 10,000 cells/well in 96-well plates containing RPMI-1640 medium and treated with apigenin (5, 10, and 20 µM) for 48 hours. Following fixation with ice-cold 10% trichloroacetic acid (TCA) and staining with 0.04% sulforhodamine B (SRB, Sigma), unbound dye was removed by rinsing with TCA. Bound SRB was then solubilized with 10 mM Tris-base, and after adding 100 µL of dimethyl sulfoxide (DMSO, Merck), absorbance was measured at 510 nm using a microplate reader.

Wound Healing Assay

SNU449 cells were seeded in a 60 mm cell culture petri dish and cultured until they reached 80% confluence. A scratch

was made in the cell monolayer using a sterile 100 µL pipette tip, and the cells were washed twice with PBS to remove debris. The cells were then treated with 5 µM Apigenin for 48 hours, and images of the scratch were captured at specified time points using a cell imager (Leica, Paula). The wound healing area was quantified using ImageJ, and the wound closure rate was calculated.

Colony Formation Assay

SNU449 cells were seeded in 6-well plates at a density of 1,000 cells per well, incubated overnight, and treated with 5 µM Apigenin for 48 hours. The medium was then replaced with fresh RPMI-1640, and it was changed every 2-3 days. The cultures were washed with PBS, fixed with methanol: acetic acid (3:1) for 5 minutes, stained with 0.5% crystal violet in methanol for 15 minutes, and washed with water. Colonies were counted, and the plating efficiency (PE) and surviving fraction (SF) were calculated using the following formulas:

PE = (number of colonies formed/number of cells seeded)*100

SF = (PE of Apigenin-treated cells/PE of control cells)*100

Statistical Analysis

The normal distribution of the variables was evaluated using the Shapiro-Wilk Test due to the small sample size. Continuous variables were expressed as median and interquartile range (IQR). The evaluation of the colony formation assay was explained previously. The Continuous variables were compared among the control and apigenin-treated groups using the Mann-Whitney U test. Comparison of the responses to different doses of apigenin in cell survival and SRB assays was performed using the Kruskal-Wallis Test for multiple group comparisons. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences software version 27 (SPSS v.27) (IBM, Armonk, NY, USA).

Results

Cell Viability Assay

The IC50 of apigenin was determined using the MTT assay. Results indicated that 5 µM at 72 hours was the minimum effective dose. The absorbance values for the 5 µM apigenin-treated and control groups were 0.581 (IQR: 0.26) and 0.67 (IQR: 0.049), respectively (p>0.05) (Fig. 1A), demonstrating a lack of statistically significant difference.

SRB Assay

We confirmed apigenin's effects on SNU449 cell survival using the SRB assay. We tested null (untreated), 5 µM, 10 µM, and 20 µM doses. The SRB assay demonstrated no statistically significant difference in cell viability between cells treated with various apigenin doses and the untreated control group (0.54 [IQR: 0.07] vs. 0.381 [IQR: 0.365]; p>0.05) (Fig. 1B).

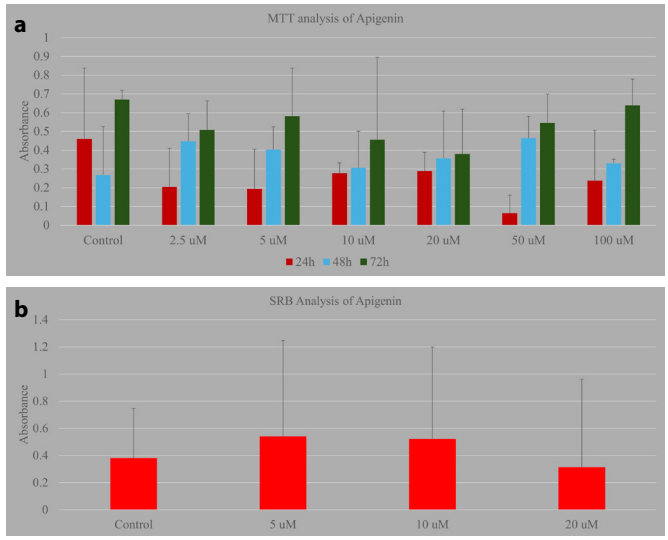

Figure 1. Summary of the results of the cell survival and SRB assay. **(a)** Dose-dependent changes in cell survival are seen. **(b)** SRB assay showing the dose-dependent changes in proliferation in SNU449 cell lines. Abbreviations: SRB: Sulforhodamine B.

Wound Healing Assay

The wound healing assay showed no significant difference in wound closure between the apigenin-treated and control groups. Wound areas at 96 hours were 528,366 (IQR: 691,200) µm^2 and 528,861 (IQR: 523,150) µm^2, respectively (p>0.05) (Fig. 2A). Wound closure rates were also similar (59.5 [IQR: 36.9] % vs. 59.75 [IQR: 15.4]%; p > 0.05) (Fig. 2B). Although the wound area appeared larger in the apigenin-treated group at 96 hours (Fig. 2C), this difference was not statistically significant.

Colony Formation Assay

The colony formation assay revealed a modest difference between apigenin-treated and untreated cells. The plating efficiency (PE) was 4.65 for control cells and 3.45 for apigenin-treated cells, resulting in a surviving fraction of 74.15% in the treated group compared to the control. These results are shown in Figure 3A-D.

Discussion

We have evaluated the effects of apigenin on the biological behavior of HCC. We have found that although it reduces the proliferative and survival capabilities of HCC cell-line SNU-449, this did not reach statistical significance. This can be interpreted as a lack of efficacy of

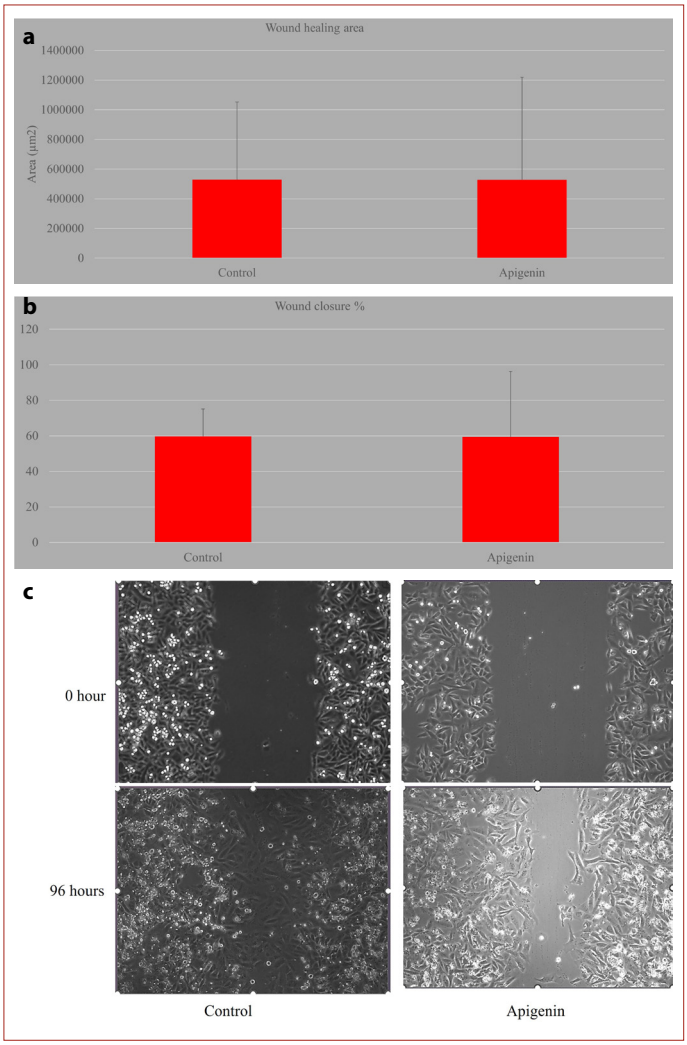

Figure 2. The results of the wound healing assay are summarized. **(a)** Wound surface area is shown. **(b)** Wound closure rates are summarized. **(c)** The figure shows the changes in the streak width of the study groups.

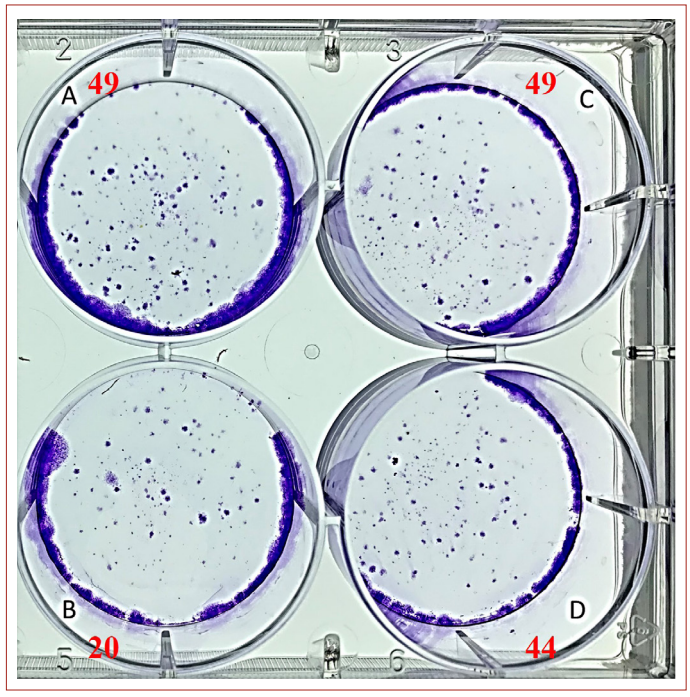

Figure 3. The results of the colony formation assay included the control and the apigenin-treated group. **(a)** and **(b)** Apigenin treated group at the end of the observation. **(c)** and **(d)** Control group at the end of the observation.

apigenin in the HCC treatment. On the other hand, it may show its effects by interacting with components of the tumor microenvironment.

Apigenin is a flavonoid that is mostly present in fruits and vegetables like parsley, celery, celeriac, and others. Since ancient times, apigenin has been used to appraise many ailments like tremors, sleep disorders, and anxiety.[14] Preclinical studies illustrate the usefulness of apigenin in the prevention as well as treatment of peri-operative stress challenges. Pre-clinical in-vitro as well as in-vivo investigations confirm the anti-tumor potentiality of apigenin in various cancers. Several studies note that apigenin can curb growth, as well as metastasis of hepatocellular carcinoma cells.[15] HCC is the fundamental kind of liver malignancy with an overall grim ending. Apigenin exhibits anticancer capability through incentives of apoptosis and autophagy in HepG2 and considerable growth suppression of HCC in HepG2 and Huh-7. In addition, apigenin can recover the anti-cancer effect of chemotherapeutic agents such as doxorubicin and Sorafenib in a mice model as well as in HepG2 and Huh-7 cells.[16] Moreover, apigenin and its unique analogs are competent to cut down the activity of multiple drug resistance mediated CYP3A4, which is impressive in improvement of the oncological treatment effect of doxorubicin.[9] Cellular alarm and demise or programmed cell death routes are accountable for the development as well as replacement tissues and suppression of cells and carcinogens bacteria. Therefore, apoptotic cell demise is vigorous against growth-propagating impact as compared to mortal natures. In diseased cells, apoptosis refuses to work correctly and transmutes into tumor progression. Necrosis is unprogrammed with disastrous results and deteriorates debris contents are discharged in the surroundings and starting inflammation.[14-16] As a result, vicious cell endeavors are developed. In any case, ATP-deficient surroundings necroptosis or programmed necrosis can be activated. In the direction of the specific genetic condition of HCC type and grade of "tumor-stage-dependent," specialists are not perpetually capable of disposing of efficacy and prevention. The bioactive plant constituent, apigenin, is located in a diversity of green provisional, cereals, and therapeutic herbs.[19] Api is a nontoxic cure with originating health

profits. The treatment of Hep3B cells with Apigenin significantly concentrated the caused demise. Hence, findings present that apigenin was prosperous in fostering the API-enhanced API-induced cell committal as well as necroptosis in Hep3B cells. On this account, Apigenin exhibited the API-induced necroptosis toxic aftermath as well as emerging it as an ally with chemotherapeutic drugs in liver cancer cure.[14-16] We have not found such effects of apigenin on SNU449 cell lines. However, what we could confirm was a modest reduction of proliferation.

There is much interest on dietary interventions with polyphenols as a new strategy in the prevention of cancer.[20] Bioactive compounds of plant origin, such as flavonoids, reduce the incidence of cancer. Epidemiological evidence suggests that a diet rich in flavonoids from fruits and vegetables may be associated with a reduced risk of lung cancer and potentially HCC.[19,20] HCC cell lines representing different genetic backgrounds were exposed to physiologically relevant apigenin levels to assess effects on cell proliferation, cell cycle, and superoxide radical formation.[21] Mouse and zebrafish HCC models were established and exposed to apigenin to evaluate effects on HCC progression, oxidative stress, and immunoediting.[22] The combinatorial effect of apigenin with current chemical HCC therapies was analyzed in HCC cell lines and zebrafish.[22] Our results suggest that the biological effects of apigenin should be together with the tumor microenvironment of HCC.

Chemotherapy is one of the most common cancer treatments that employ chemical compounds that can kill, or limit the growth of, cancer cells. Currently, chemotherapy is the treatment of choice for many types of cancers.[19-22] However, potent antineoplastic chemicals harm healthy tissues, especially tissues with high cell turnover rates, such as the gastrointestinal tract, hair follicles, and bone marrow. Moreover, long-term chemotherapies harshly affect the liver, kidneys, and heart, which are responsible for detoxifying the body from potentially toxic agents.[19-22] Many chemotherapeutic agents have a very narrow therapeutic window due to their low bioavailability, rapid metabolic clearance, and considerable toxicity. Inhibition of certain metabolic enzymes by some phytochemicals has previously been reported.[23] Since many clinically used drugs, including chemotherapeutic agents, are substrates for these enzymes, chemopreventive phytochemicals that inhibit metabolic enzymes may enhance the action of these drugs by altering their pharmacokinetics. So, the use of specific phytochemicals in combination therapy with specific chemotherapeutic drugs can result in a significant increase in drug efficacy and, at the same time, it may lead to a reduction in side effects.[24] Among the co-administered phytochemicals, flavonoids have an advantageous effect on the pharmacokinetic parameters of drugs. Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth leading cause of cancer-related deaths globally. Surgical resection and liver transplantation offer curative treatment for early-stage HCC.[23,24] Chemotherapy is considered a palliative way to prolong the patient's life by 2–3 months. In many hepatocellular carcinoma patients, their impaired liver function eliminates the option of surgical intervention.[23,24] The overall 5-year survival rate is about 5%. Chemotherapeutics with plant extracts, including certain compounds, may be used to inhibit the growth of cancer cells. Certain compounds potently inhibit parasite hepatic cell growth by blockading the cell cycle and inducing apoptosis.[23,24] These compounds produce a concentration-dependent decrease in parasitic hepatic cell survival, with elevated values from 20 μM to 80 μM. In combination with certain inhibitors, these compounds synergistically inhibit the hepatic cell growth of parasites. These compounds suppress hepatocellular carcinoma cell growth by targeting specific genes. By targeting these genes, they abate hepatocellular carcinoma cells' migrating and invading abilities. The repurposing of these compounds, together with the current treatment protocol, might improve the effectiveness.[23,24] Apigenin radiosensitizes HCC by promoting radiation-induced apoptosis and G2/M phase cell cycle arrest, and inhibiting AP-1 and HIF-1α.[25,26] However, the potential of apigenin to enhance the radiosensitivity of tumor cells has yet to be sufficiently studied.[25,26] We have concentrated on the antiproliferative effects of apigenin and we could not prove our hypothesis. We plan to use apigenin in combination with various chemotherapeutics to search the synergistic effects.

Our study is an in vitro study evaluating the effects of apigenin on the proliferation of HCC cell line SNU449. This is the most important limitation of the present study. We did not consider the component of the tumor microenvironment. Therefore, in the future, we intend to use apigenin alone and in combination with antitumor agents in animal models to evaluate the mechanism of action of this flavonoid.

Conclusion

There is a rapidly emerging body of in vivo and ex vivo data from animal-based studies that supports the contention that apigenin has real promise as an anti-cancer agent. Essentially, these studies are now broad enough in scope to suggest possible mechanistic themes for future investigations and to justify progression to human applications. However, to provide an effective foundation for undertaking randomized controlled trials based on these observations, it is important to establish parameters for dosing frequency, total dosage, bioavailability, effective

serum concentration, routes of administration, and efficacy in preventing, reversing, or modifying dysplasia and neoplastic disorders. Efficient translation from the bench to the clinic necessitates comprehensive efficacy studies with well-characterized standardized products and dosing regimens. Randomized controlled trials must be based on validated marker metrics showing a significant reduction in tumor burden or tumor progression. Highly relevant to the digestive tract, such trials also require validated data on efficacy in specific cell types and a comprehensive understanding of interaction with key signaling pathways that ultimately determine enzyme activation and cell progression. Experimental design must also involve the interrogation of the therapeutic window (i.e., the interval between optimal dose for effective treatment and the maximum dose that can be taken before adverse effects occur). Comprehensive understanding of apigenin metabolism and a wider series of well-conducted preclinical studies that are directly translatable into clinical settings are then the preclinical requirements. At the very least, all of these factors should be established in the literature before embarking on phase I clinical trials, the primary goal of which would be validation of the safety of apigenin exposure at the anticipated doses. Currently, we could only prove a modest antiproliferative effect of this flavonoid.

Disclosures

Ethics Committee Approval: None.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

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Case Report

Primary Kidney Lymphoma Mimics Renal Cell Carcinoma in Preoperative Liver Transplant Patient: Postoperative Challenge – A Case Report

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Abstract

Cancer screening is a critical component of the pretransplantation process. However, in patients with deteriorating liver function, the focus may shift towards acquiring a functioning liver, potentially delaying cancer evaluation.

A 42-year-old male with primary sclerosing cholangitis (PSC), CTP score C10, and MELD score 19 was evaluated for liver transplantation (LT). Preoperative imaging revealed two lesions in the upper pole of the left kidney, raising concern for malignancy. After discussion at a uro-oncology meeting, the decision was made to resect the lesions post-LT. On postoperative day 5, the patient underwent entero-enterostomy revision and resection of the renal lesions due to melena. Pathological analysis confirmed Burkitt lymphoma/high-grade B-cell lymphoma-NOS. The patient completed four cycles of chemotherapy and remained free of recurrence by the second postoperative year.

This case underscores the importance of cancer screening in LT candidates and highlights the need for biopsy when radiologic suspicion arises. Management should be individualized, considering both liver disease severity and malignancy, with a tailored treatment approach.

Keywords: Post-transplantation lymphoproliferative disease, Ebstein-Barr Virus (EBV), Burkitt Lymphoma, Liver transplantation

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Screening for systemic cancer is an important part of both liver transplant recipient and donor preparation. During recipient preparation, if an extrahepatic malignancy is detected, an individualized treatment protocol should be established based on the type of malignancy and the severity of the patient's liver disease. Depending on the severity of the malignancy and the 5-year life expectancy, liver transplantation can be planned in some cases without a post-treatment waiting period, while in others it has been adopted by many transplant centers to remove the patient from the transplant list and perform transplantation after the follow-up periods recommended by the guidelines for malignancy treatment.[1] Institutional Review Board (IRB) approval was not required by IRB for the preparation of this report but written informed consent was obtained from the patient for publication.

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Non-Hodgkin lymphoma (NHL) accounts for about 90% of all lymphoma cases, with 85-90% arising from B lymphocytes and the remainder derived from natural killer (NK) and T lymphocytes.[1, 2] 25-35% of NHL cases arise from extra-nodular areas such as the small intestine, skin, and stomach. Secondary renal involvement may be observed in 30-60% of disseminated NHL cases; however, primary renal lymphoma (PRL) is rare and accounts for less than 1% of all renal masses.[3-5] PRL cases can be mistaken for RCC due to the peripheral localization of the mass and similar radiological findings. Early-stage RCC is a malignancy with a 5-year survival rate of over 95% when detected. However, due to different tumor biology and outcomes, pre-transplantation approaches vary between these two patient groups.[2]

In this report, we present an instance of LT for a patient incidentally diagnosed with high-grade PRL. Institutional Review Board approval was not required for the preparation of this report but written informed consent for publication was obtained from the patient.

Case Report

A 42-year-old diagnosed with history of Primary Sclerosing Cholangitis (PSC) was admitted to our center for LT for further decompensated liver cirrhosis. Child-Turcott-Pugh score was C10, and a MELD score was 19.

The physical examination revealed a poor general condition, widespread muscular atrophy, hepatosplenomegaly, jaundice, and Grade 2 hepatic encephalopathy. Preoperative radiological evaluation identified two lesions, measuring 23 mm and 8 mm, located on the lateral and medial sides of the upper pole of the left kidney, respectively, suggesting early-stage renal cell carcinoma (RCC). (Fig. 1) During the case-based Uro-Oncology meeting, it was agreed that removal of the masses would be appropriate following liver transplantation (LT) and normalization of liver functions, given the advanced liver disease, significant thrombocytopenia, and coagulation disorder.

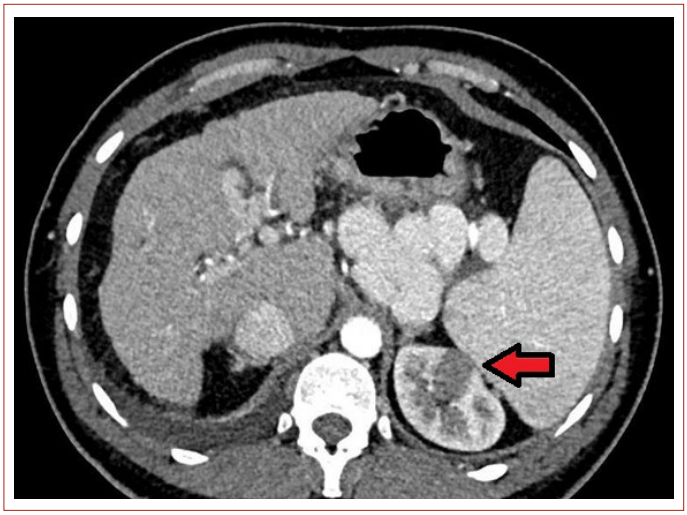

Figure 1. Left Kidney Upper Pole Lateral RCC, CT image.

The patient underwent a liver transplant of the right lobe and a splenectomy, featuring a single arterial anastomosis, a single portal vein anastomosis, and multiple hepatic vein anastomosis, receiving the organ from his sister who has the same blood type. To address the bile duct compromised by underlying PSC, a Roux-en-Y hepaticojejunostomy was performed for the biliary anastomosis.

On the postoperative day 5, despite medical treatment and endoscopic interventions, gastrointestinal bleeding persisted and entero-enterostomy revision was needed. Due to normalization of the liver functions and coagulation parameters, excision of renal masses was also performed. The patient was discharged on the postoperative 20th day uneventfully.

The pathological examination of the left-sided masses identified them as Burkitt lymphoma / High-grade B-cell lymphoma, not otherwise specified (NOS). The analysis showed B-cell neoplastic infiltration with necrotic changes, pronounced kappa light chain clonality, and high proliferative activity. Immunomarker assessment was revealed as following Leukocyte common antigen (LCA) (+), cluster of differentiation (CD) 79a (+), paired box protein (pax) 5 (+), CD19 (+), CD3 (-), CD20 (+), kappa light chain (+), Lambda Light Chain (-), CD10 (+), B-cell leukemia/lymphoma (BCL) 6 (+), BCL-2 (-), CD5 (-), cellular myelocytomatosis oncogene (CMYC) (-), Ki-67 95%

Hematology and oncology board suggested positron emission tomography (PET) scan and, no further lesion was detected. 4 cycles of adjuvant Rituximab, Cyclophosphamide, Doxorubicin and Prednisolone regimen were administered. The patient is followed up in the 2nd postoperative year of the procedure with normal liver functions and no recurrence.

The second-year follow-up evaluations of the patient after 4 cycles of adjuvant Rituximab, Cyclophosphamide, Doxorubicin and Prednisolone (R-CHOP) regimen was tumor free and liver functions were also normal.

Discussion

There is an increase in incidence of PRL which correlates with the increasing incidence of NHL related to development and availability of diagnostic modalities. There is a male predominance with being reported up to 1.6:1.[5] Most cases of PRL reported in the literature are unilateral, with bilateral renal involvement comprising less than 20% of the cases. The pathophysiology of PRL has been debatable as kidney is not a lymphatic organ and the origin of the lym-

phoma cells is not well clarified, and it is thought to be origin of the PRL is linked to lymph nodes of the renal sinus or the lymphatics of the renal capsule. Furthermore, PRL may be associated with history of chronic inflammation and pyelonephritis, Epstein-Barr Virus, and autoimmune diseases.[5, 6] On the other hand, extra-nodal NHL presenting as a solid lesion mimics other types of solid organ malignancies before histopathologic examination is performed, diagnosing PRL remains to be a challenge.[7]

Stallone et al. have proposed three diagnostic criteria for PRL: lymphomatous renal infiltration, no extra-nodal localization and non-obstructive unilateral/bilateral enlargement.[6] The diagnosis of PRL and its differentiation from other primary renal malignancies including RCC is critical especially in terms of further treatment. Ultrasonography may detect homogeneous, hypoechoic lesion in cases of PRL, however the imaging study of choice is computed tomography (CT) which demonstrates hypovascular masses with homogeneous attenuation unlike heterogeneity of RCC. Hypovascularity of PRL observed using color doppler also differs from the hypervascular pattern of RCC.[8] On CT scan, PRL typically presents with the following patterns: presence of multiple and bilateral hypovascular renal masses, presence of peri-renal disease, diffuse renal infiltration and renal invasion from contiguous retroperitoneal disease.[9] However, CT findings of PRL remain to be unspecific and histopathologic examination is considered gold standard in the diagnosis, with the most common histology type being the diffuse large B cell lymphoma (DLBCL) followed by marginal zone lymphoma. PET scan may be also useful in the diagnosis of PRL as NHL is intensely FDG-avid while RCC may not show intense FDG uptake.[10] Accurate diagnosis of PRL is vital as the survival and renal function has improved in PRL patients owing to the modern chemoradiotherapy, while RCC is managed surgically in most cases.[11] Histopathologic evaluation of PRL is also essential for grading which is the primary factor determining further management.

The use of CHOP (Cyclophosphamide, Doxorubicin, Vincristine and Prednisolone) has been reported for PRL in various case series, however it should be acknowledged that addition of rituximab could improve the survival.[12] The role of surgery in PRL is controversial, however, in more than 90% of case reports, nephrectomy has been performed with the differential diagnosis of RCC.[13] Due to being the most common subtype of PRL diffuse large B cell lymphoma, overall mortality of PRL despite new advances in treatment remains to be high. Delayed diagnosis also contributes to the poor survival. In their study of 599 primary renal lymphoma patients Taneja et al. report a median overall survival of 115 months. Overall male gender, stage III-IV, DLBCL histology and advanced age are considered as poor prognostic factors.

In connection with this information, in cases of lymphoma, it is recommended to wait 2 to 5 years after remission, as lymphoma relapse often occurs in this time frame.[14] In contrast, in patients with early stage RCC, there is no specific waiting period for liver transplantation. Depending on the severity of liver disease, even elective kidney surgery may be performed when liver functions return to normal after liver transplantation.[15]

In our patient, preoperative evaluations revealed two renal masses in the left kidney, which were initially thought to be consistent with renal cell carcinoma (RCC). The diagnosis was misled by the rarity of primary renal lymphoma and the more common occurrence of RCC. Fortunately, the masses were successfully removed via laparotomy, and an early diagnosis of "lymphoma" was established, leading to a good response to chemotherapy. However, had it been known that the renal mass was lymphoma, it would have been a contraindication for liver transplantation (LT) according to clinical guidelines, and the transplantation would not have been planned.

Furthermore, if preoperative biopsy or increased suspicion towards lymphoma were present, transplantation process would be reconsidered as defined by the guidelines with combined decision of transplantation team and haemato-oncology team. On the other hand, transplantation team should consider making a long-term transplantation plan for this specific type of patient group via close follow-up of their hematological progress whether remission is achieved without active disease in between 2-5 years. Individualized, risk-assessed and multi-disciplinary approach in patients with dire necessities for liver transplantation in this patient group is also a must to achieve both hematological and transplantation success. Risk stratification should be made more diligently in patients with more dire need of liver graft than need of hematological remission.

Cancer screening is one of the most important evaluations for liver transplant candidates. Especially in case of radiologic suspicion, tissue biopsy should be performed to establish the actual diagnosis. In addition, the severity of the patient's liver disease and the detected malignancy should be evaluated on a patient-by-patient basis, risk analysis should be performed, and the most appropriate treatment plan should be organized.

Disclosures

Informed consent: Written informed consent was obtained from the patient for the publication of the case report and the accompanying images.

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Case Report

Coma Blister Mimicking Necrotizing Fasciitis in a Liver Transplant Patient: A Case Report

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Abstract

Coma blisters (CB) are self-limiting cutaneous lesions that typically occur in patients with prolonged impaired consciousness, often due to drug overdoses such as barbiturates. They are rarely observed in liver transplant patients and can clinically mimic conditions like necrotizing fasciitis. We report the case of a 49-year-old male post-liver transplant who presented with bullous and necrotic lesions on the anterior abdominal wall, initially suspected to be necrotizing fasciitis. A skin biopsy confirmed CB, and the patient responded to corticosteroid treatment, with improvement over 20 days. The patient returned 10 months later with similar lesions, which again resolved with similar management. Early diagnosis and appropriate treatment are essential, particularly in immunocompromised patients, to distinguish CB from more serious conditions.

Keywords: Coma blisters, necrotizing fasciitis, liver transplantation

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Coma blister (CB) is usually seen as a result of the overdose of drugs such as benzodiazepines, antipsychotics, opiates, tricyclic antidepressants, alcohol, heroin, and barbiturates. It is rarely seen in patients with long-term impaired consciousness.[1,2] It has also been observed in patients with chronic renal failure, hypercalcemia, carbon monoxide poisoning, diabetic ketoacidosis, and various neurological disorders.[3] Skin lesions usually appear after 48-72 hours and resolve within 2-4 weeks.[1,2] The mechanism of CB formation is not fully understood, but timely treatment is essential. CB is a self-limiting cutaneous disease. Patients may present with complaints of pain, edema and skin necrosis in the affected area. Because it is rarely seen, clinicians may it misdiagnose as necrotizing fasciitis or cellulitis.[4] A 47-year-old male patient presented with the complaint of extensive skin necrosis on the anterior abdominal wall. We present the case because this situation is special in a patient who had a liver transplant before.

Case Report

A 49-year-old male patient who had undergone liver transplantation for Budd-Chiari 5 years ago was continuing his routine controls. Three years after liver transplantation, the patient was admitted to our clinic with ecchymosis, bullous and necrotic lesions on the anterior abdominal wall for 3 days (Fig. 1). On the physical examination, necrotizing fasciitis was considered in the differential diagnosis, and antibiotic therapy was started since there was ecchymosis in an area localized with bullae on the right side of the anterior abdominal wall and severe pain on palpation. Neurological

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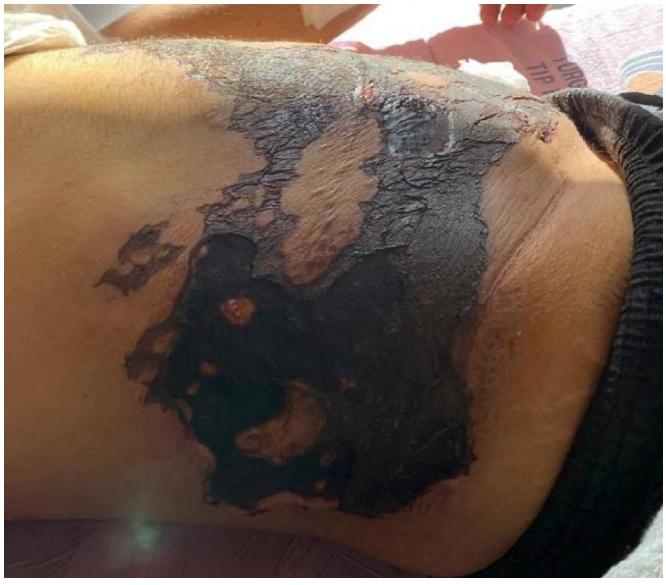

Figure 1. Picture of the patient's lesion at the time of admission.

examination of the patient with high acute phase reactant levels was normal.

There was no finding in favor of necrotizing fasciitis in the abdominal tomography of the patient (Fig. 2). The patient who had a liver transplant due to Budd-Chiari was regularly using tacrolimus, warfarin, ursodeoxycholic acid, and esomeprazole. The patient's tacrolimus level was normal (7.6: 5-20), and his INR was 1.6. A biopsy was taken from the patient's lesion and the patient was consulted with the dermatology department. Purpura fulminans, warfarin necrosis, bullous pyoderma gangrenosum, ecthyma gangrenosum, necrotizing fasciitis were considered in the differential diagnosis. In addition to antibiotics, methylprednisolone 40 mg and the wet dressing were started as treatment. In addition, the immunosuppression dose taken by the patient was decreased and continued.

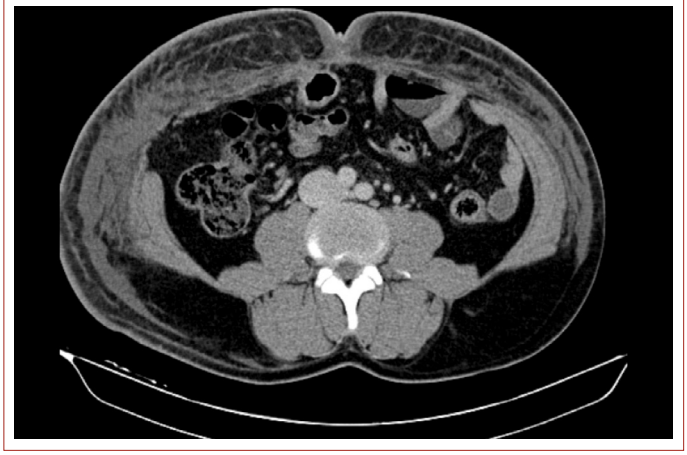

Figure 2. Abdominal tomography of the patient at the time of admission.

The patient's complaints regressed after 7 days and the color of the necrotic areas began to improve (Fig. 3). Heparin treatment was started for the patient using Coumadin. And the dose of methylprednisolone was gradually reduced. The wound dressing was continued with antibiotic creams. After 20 days of hospitalization, necrotic tissues were debrided and wound dressing of the ulcerated area was continued. After 4 weeks, the pathology result was reported as a coma blister. And the patient's methylprednisolone and the antibiotic cream dressing were continued. After 7 days, the methylprednisolone treatment was stopped. The patient was discharged on the 40th day.

10 months later, the patient came back to the clinic with complaints of redness, bruising and pain in the right thigh (Fig. 4). The necrotic lesion, which was severely painful on palpation, resembled the lesions 10 months ago. The patient was hospitalized and after 1 week of antibiotic, steroid and anticoagulant treatment, the lesion resolved and the patient was discharged. No new lesion developed in the follow-up of the patient (Fig. 5).

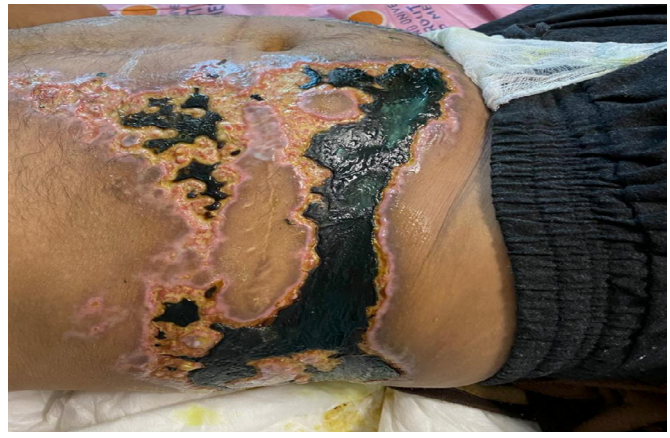

Figure 3. Picture of the patient's lesion during treatment.

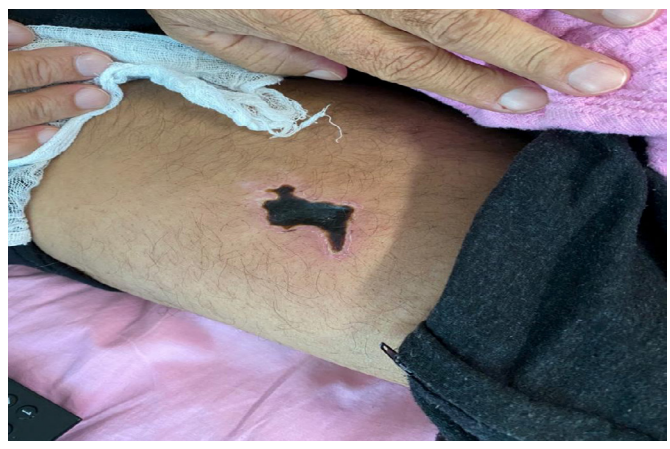

Figure 4. Picture of the lesion developing on the patient's thigh.

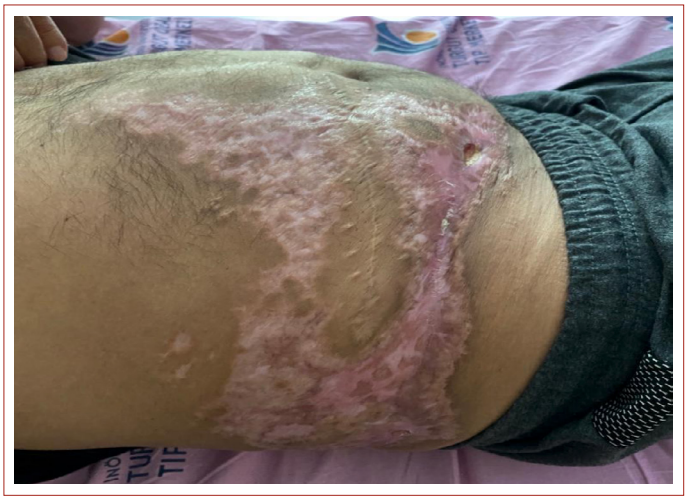

Figure 5. Picture of patient's response to treatment 6 months after treatment.

Discussion

Coma blisters are self-limiting lesions that occur in a comatose state for a variety of reasons but are most commonly associated with barbiturate overdose. Examination of the skin biopsy specimen shows the characteristic presence of eccrine sweat duct necrosis.[5] When CB was first described in the medical literature in 1965, it was reported that CBs were caused by barbiturate poisoning.[6] Later, it was thought that CB was caused by pressure in patients with impaired consciousness and ischemia in places where the pressure was high. However, due to its distribution and localization, this situation could not be explained by increased pressure or vascular pathology. While it has been suggested that some toxic effects of drugs may play a role, the relationship between CB and any specific drug has not been proven. He demonstrated that these skin lesions caused by barbiturates can be distinguished by the specific histological finding of sweat gland necrosis. They can usually be seen in both pressurized and non-pressurized areas on the extremities and trunk.[7]

There are also studies reporting damage to sweat glands and ducts and related tissue hypoxia as the main mechanism of CBs.[8] CB is located on pressure areas, suggesting that dermal pressure injury is an important underlying mechanism. However, they have also been reported to affect non-pressure sites, suggesting that additional mechanisms may be involved, including drug toxicity, hypoxia, hypothermia, metabolic acidosis, and immune system disorders. In the differential diagnosis, pemphigoids may include epidermolysis bullosa, bullous drug eruptions, bullous diabeticorum and postburn bullae.[2]

CB, which is not well defined in the literature, should be suspected in patients with severe pain, as it may cause irreversible nerve and muscle damage.[4] Necrotizing fasciitis is a serious, potentially fatal soft tissue infection that progresses rapidly and can develop into septic shock.[9] 70-90% of these patients are polymicrobial and they are accompanied by various comorbidities such as diabetes mellitus.[10] These two diseases can mimic each other. The etiology, clinic and treatment of coma blister have been discussed above. Necrotizing fasciitis is a life-threatening bacterial infection that rapidly destroys subcutaneous tissue that reaches the muscles. While coma blister is usually painless and can be healed with simple wound care, necrotizing fasciitis causes severe pain and requires surgical debridement and broad-spectrum antibiotic therapy. If not treated early, necrotizing fasciitis can lead to serious consequences such as sepsis, organ loss and death, while coma blister usually does not cause such complications.[11]

A biopsy was taken from the lesion that clinically and radiologically mimicked necrotizing fasciitis in our patient, and symptomatic treatment was started. As a result of the skin biopsy, the diagnosis of CB was confirmed.

Because CB lesions are self-limiting, a broad-spectrum topical antibiotic cream is sufficient to prevent secondary infection of ruptured bullae. Most bullae heal within two weeks with the regeneration of clinically intact normal skin.[7]

Conclusion

Coma blisters are lesions that can accompany various neurological diseases. Although they are most commonly associated with barbiturate overdose, they can also be seen in coma due to other etiologies. Blisters develop 48 to 72 hours after the onset of unconsciousness. Because liver transplant patients are immunosuppressed, early diagnosis and treatment for coma blisters are important.

Disclosures

Informed Consent: Written informed consent was obtained from the patient for the publication of the case report and the accompanying images.

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Letter to the Editor

The Importance of Conventional Angiography in the Preparation for Living Donor Hepatectomy

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To Editors;

In Türkiye and other far east countries, where deceased donor donation rates are insufficient to meet the needs of patients on the transplantation waiting lists, this issue is being solved through living donor liver transplantation (LDLT). LDLT offers advantages compared to deceased donor liver transplants, such as direct organ procurement, the ability to perform the procedure under optimal conditions, and a reduced rate of primary organ dysfunction due to short cold ischemia time.[1] However, LDLT cannot be performed for every recipient who applies with a living donor candidate due to donor and recipient incompatibilities, and only 30-55% of donor candidates are eligible as suitable donors.[2] These incompatibilities can be broadly categorized under three headings: blood group incompatibility, volume mismatch, and anatomical incompatibility. Blood tests and dynamic cross-sectional imaging methods are the most crucial stages of living donor preparation. In cases where routine examinations fail to clarify certain conditions, advanced investigations, including conventional angiography or surgical exploration, may be required for the donor candidate. The evaluation of the vascular anatomy of the donor candidate's liver is largely sufficient with three-phase multislice dynamic computed tomography (CT). In cases where arterial anatomy cannot be clearly assessed using CT, conventional angiography is crucial for defining the arterial anatomy, as it is of vital importance for both the donor and the recipient.

A 54-year-old male patient, who was indicated for liver transplantation due to cirrhosis developed on the basis of chronic hepatitis B, had a suitable donor in his 28-year-old son. However, the arterial anatomy of the donor's liver could not be clearly evaluated using dynamic CT. Conventional angiography was performed on the donor candidate to delineate the hepatic arterial anatomy. The angiography revealed that the liver was supplied by hepatic collateral circulation due to chronic stricture in the celiac trunk (Fig. 1).

Since the current situation was unsuitable for both right and left hepatectomy, the donor candidate could not be added to the liver paired exchange pool and was disqualified as a living donor after being informed that they were not a suitable candidate for living donor hepatectomy. As the donor candidate had no gastrointestinal symptoms, no intervention was performed on the celiac trunk. The re-

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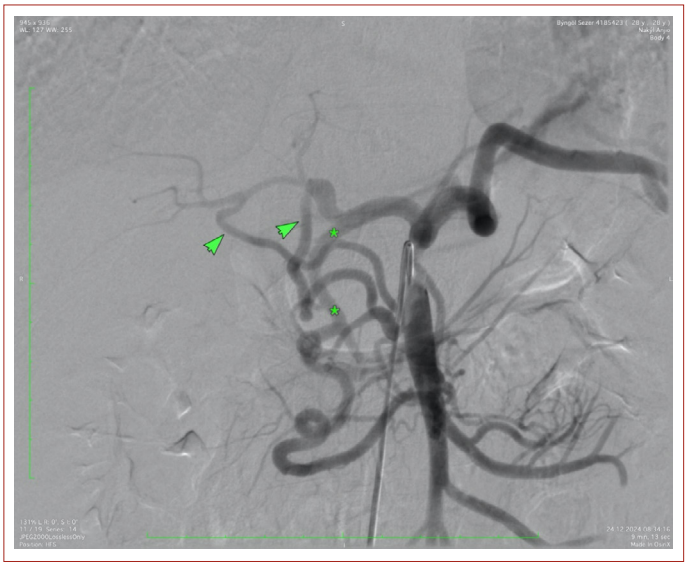

Figure 1. In selective superior mesenteric artery (SMA) injection, the celiac trunk is seen filling retrogradely through the GDA (asteriks) where the pancreaticoduodenal arcade is prominent, and two distinct arteries (communicating channels) connecting from the SMA to the right hepatic artery and hepatic propria (arrowheads) are seen. The anatomical course of these communicating channels is nearly similar to that of the hepatic artery variant that originates from the SMA. Therefore, these channels could be the embryonic remnants that form aberrant hepatic arteries.[3]

cipient, who had compensated cirrhosis with a MELD-Na score of 16, was advised to find another donor and was discharged. The patient later presented with another donor candidate, and a successful LDLT was performed. The donor and recipient were discharged without complications, following their intensive care and general ward processes.

As a result, if the donor hepatic artery anatomy cannot be clearly evaluated with dynamic cross-sectional imaging, invasive conventional angiography should be performed without hesitation. Proceeding to surgery without clearly defining the anatomy may lead to donor abortion, unnecessary anesthesia, and surgery.

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From the Congress President

I believe that publishing the oral presentations introduced at the 12th National Gastroenterological Surgery and 2nd Liver Transplantation Congress, organized by the Society of Gastroenterological Surgery and Society of Liver Transplantation on November 7-9, 2024, in Malatya, in JILTI will be beneficial to readers in terms of enlightening topics. In this issue, we have allocated space for these oral presentations, which are related to the gastrointestinal system, including hepatobiliary subjects.

President of the Congress

Prof Dr Sezai Yilmaz MD, FACS

Inonu University

Director of Liver Transplantation Institute

Oral Presentation Sessions

Session Chairs

Prof. Dr. Sami Akbulut, Assoc. Prof. Dr. Veysel Ersan

1. Difficult Harvesting, HIATT Type 5, Arterial Anomalous Case
   Mehmet Serhat Ozaydin, Mehmet Can Aydin, Emil Huseyinoglu, Osman Serhat Guner, Mehmet Akif Ustuner
2. Our 1.5-Year Single-Center Experiences in Liver Transplantation
   Eray Can Akinci, Osman Serhat Guner, Emil Huseyinoglu, Gokhan Garip, Mehmet Can Aydin, Ozkan Balcin, Murat Sayilgan, Mehmet Akif Ustuner
3. Adult Living Donor Liver Transplantation Experience of Diyarbakır Dicle University Organ Transplant Center
   Mehmet Yilmaz, Murat Sevmis, Hatice Gulsen Yilmaz, Ulas Aday, Abdullah Oguz, Mehmet Veysi Bahadir, Muhsin Kaya, Kendal Yalcin, Ayhan Kaydu
4. Is the Use of Transanastomotic Internal Stents in Biliary Reconstruction a Reliable Option to Reduce Biliary Complications?
   Mehmet Yilmaz, Murat Sevmis, Hatice Gulsen Yilmaz, Ulas Aday, Abdullah Oguz, Mehmet Veysi Bahadir, Muhsin Kaya, Kendal Yalcin, Ayhan Kaydu
5. Our Experience with Challenging Liver Transplantation Cases in Our Center
   Ali Baybars, Osman Serhat Guner, Ahmet Aglarin, Kemal Aslan, Mehmet Akif Ustuner

Prof. Dr. Huseyin Ciyiltepe, Assoc. Prof. Dr. Abdullah Senlikci

6. Superior Mesenteric Artery Syndrome, Hiatal Hernia, and Nutcracker Syndrome Combination: Case Presentations and Literature Review
   Hakan Yirgin, Muhammed Bayram, Yasir Kesgin, Yunusemre Tatlidil, Ozgur Gangal, Ilhan Gok
7. Multiple Complicated Enteroenteric Intestinal Fistula Due to Crohn's Disease: A Rare Case Presentation
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1. Difficult Liver Graft Harvesting: A Case With Hiatt Type 5 Arterial Anomaly

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Introduction:

To present our experiences with a case involving HIATT Type 5 anomaly encountered during the harvesting phase of cadaveric liver transplantation.

Methods:

The case was completed after appropriately classifying the anomaly identified through pre-harvesting imaging studies and perioperative exploration findings.

Results:

A 34-year-old male patient with no known comorbidities was identified as a cadaveric donor following brain death due to head trauma. Harvesting was planned.

Pre-harvesting tomography revealed that the main hepatic artery originated from the superior mesenteric artery (SMA), while only the splenic artery and left gastric artery originated from the celiac trunk.

Discussion:

Unexpected anatomical variations may be encountered during donor harvesting. Arterial anatomical variations are particularly common and have been classified in various ways. In our clinic, we use the Hiatt classification. Preoperative abdominal CT imaging is essential for understanding these variations when available. For donors without imaging, it is crucial to anticipate possible variations and adapt the procedure accordingly.

Keywords: Liver transplantation, harvesting, Hiatt classification, arterial anomaly

2. Our Single-Center Experience In Liver Transplantation: Acheivements of a Newly Established Center

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Introduction:

As a newly established center in liver transplantation, we aimed to present our experiences in liver transplant surgeries and patient follow-ups over an 18-month period.

Methods:

Over 18 months, 12 cadaveric liver transplants were performed. The perioperative and postoperative conditions observed and applied to these 12 patients were recorded. The patients' characteristics, evaluated through laboratory and imaging tests, are presented in the table.

Results:

Organs were obtained from cadaveric donors and transplanted to 12 patients (4 females, 8 males). The median age of operated patients was 56 (21-72 years). The median operation time was 640 (460-990) minutes. A stent was used during bile duct anastomosis in one case. No major intraoperative complications were observed. In the postoperative period, patients had a median intensive care stay of 3 (2-11) days and a median hospital stay of 21 (10-33) days before discharge (Table 1). Biliary complications were observed in two patients, and PTK catheters were placed. No rejection was observed in any patient. Mortality did not occur in this series.

In the long term, only one patient underwent reoperation due to umbilical hernia development.

Discussion:

Studies on liver transplantation etiology show the highest rate belongs to viral hepatitis (primarily HBV), followed by cryptogenic cirrhosis, HCV, and alcohol-related cirrhosis. Similarly, HBV-induced cirrhosis was the most common etiology in our series, followed by NASH, autoimmune hepatitis, and cryptogenic cirrhosis.

Studies report bile leaks in 5-10% of patients. In our series, bile leakage was observed in one patient (6%), and a PTK catheter was placed. This rate is consistent with the literature. The bile duct stricture rate in the literature is 13%, while our study observed a 6% rate, aligning with the literature.

Studies indicate mortality rates below 10% after liver transplantation. No mortality was observed in our series.

Keywords: Liver, Transplantation, Cadaveric

Table 1. The characteristics of the patients transplanted in our institution.

	Age	Gender	Diagnosis	Duration of Operation	Perioperative Characteristics	ICU stay	Duration of stay in the Wards	Complications
1	60	K	PBS+HCC	640	-	2	12	
2	26	E	WILSON	460	-	3	15	
3	46	E	HBV	625	-	2	21	
4	52	E	HBV	990	ILIAC ARTERIAL GRAFT	2	22	INCISIONAL HERNIA
5	71	K	NASH	570	-	5	21	
6	62	K	CRYPTOGENIC	640	-	11	10	COVID
7	50	E	HCV	650	-	2	32	
8	67	E	MASLD	700	--	3	32	BILIARY LEAK
9	40	K	CRYPTOGENIC	560	-	2	13	
10	21	E	AUTOIMMUNE HEPATITIS	750	--	8	33	SEIZURES+ BILIARY STENOSIS
11	61	E	HBV	610	-	7	15	
12	62	E	ALCOHOL+ HCV	760	-	3	22	

MASLD: Metabolic Dysfunction-Associated Steatotic Liver Disease; HBV: Hepatitis B Virus; HDV: Hepatitis Delta virus; HCV: Hepatitis C virus; MELD: Model for end-stage liver disease; Covid: corona virus-associated infectious disease; ICU: intensive care unit.

3. Adult Living Donor Liver Transplantation Experience of Diyarbakir Dicle University Organ Transplant Center

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Objective

This study aims to present the results of living donor liver transplants (LDLT) performed in our institution, starting in May 2023.

Methods

The outcomes of 41 LDLT procedures conducted between May 2023 and October 2024 in our clinic were retrospectively analyzed.

Results

The indication for transplantations are summarized in Table 1. The mean age of the recipients was 48.34 years [19-67]; 17 (41%) were female, and 24 (59%) were male. The mean MELD score was 20.07 [10-33]. The mean duration of the recipient's surgery was 8.7 hours [7-12], and the median cold ischemia time of the grafts was 105.7 minutes [70-178]. The graft-to-recipient weight ratio (GWRW) was 1.01 [0.7-1.4]. Among the grafts, 40 (98.6%) were right lobe, and 1 (2.4%) was left lobe. The mean postoperative hospital stay was 13.6 days [1-28] (Table 2).

Intra-abdominal hemorrhage necessitated reoperation in one patient (2.4%) during the fourth postoperative month; this patient later died due to Klebsiella sepsis. A challenging case with Budd-Chiari syndrome required extensive recipient hepatectomy and intraoperative transfusion of 8 units of erythrocytes. This patient succumbed on postoperative day 1 due to primary graft non-function and disseminated intravascular coagulation (DIC).

Six (14.6%) patients underwent relaparotomy: five (12%) for bleeding control and one (2.4%) for arterial revision. Arterial reconstructions were performed with 8/0 Prolene sutures. Early hepatic artery thrombosis developed in two patients (4.8%), and late hepatic artery stenosis occurred in one patient (2.4%). Arterial revision was successfully performed in the other case via relaparotomy. In one patient (2.4%), stenting was required for hepatic artery stenosis at week 6. One of the patients with arterial thrombosis was treated with stenting; however, on the 10th day postoperatively, occlusion of the anterior arterial branch was observed.

Small-for-size syndrome developed in one patient, who succumbed to Klebsiella sepsis on postoperative day 35. Biliary reconstructions were performed with continuous posterior and anterior wall sutures using 6/0 Prolene over internal stents. Biloma developed in two patients (4.8%), which were resolved by percutaneous drainage on days 30 and 35.

Conclusions:

In our clinic, LDLT mortality occurred in 3 cases (7.2%) during the early postoperative period and in 2 cases (4.8%) during the late postoperative period, totaling 5 cases (12%). As a new center, 36 (88%) of our cases survived, with a mean follow-up duration of 10.2 months.

Keywords: Living Donor Liver Transplantation, Survival and Morbidity, Complications

Table 1. The indications for liver transplantation.

	n (%)
HBV	10 (24.4)
HBV+HDV	11 (26.8)
Hepatocellular carcinoma	6 (14.6)
Cryptogenic cirrhosis	9 (22.0)
MASLD	1 (2.4)
Wilson's disease	1 (2.4)
Budd-Chiari syndrome	1 (2.4)
HCV	1 (2.4)
Autoimmune hepatitis	1 (2.4)

MASLD: Metabolic Dysfunction-Associated Steatotic Liver Disease; HBV: Hepatitis B Virus HDV: Hepatitis Delta virus HCV: Hepatitis C virus.

Table 2. The perioperative characteristics of the patients.

Right lobe graft	40 (97.6%)
Sol lob graft	1 (2.%4)
GWRWR	1.0 [0.7-1.4]
Duration operation ratio	8.7 [7-12] hr
Volume of blood transfusion	2.41 [0-10] units
Duration of hospitalization	13.6 [1-28] days
Amount ascites	1.57 [0-8] L
Cold ischemia time	105.7 [70-178] min

GWRWR: Graft Weight Recipient Weight Ratio.

4. Is the Use of Transanastomotic Internal Stents in Biliary Reconstruction a Reliable Option to Reduce Biliary Complications?

Mehmet Yilmaz[1], Murat Sevmis[1], Hatice Gulsen Yilmaz[1], Ulas Aday[1], Abdullah Oguz[1], Mehmet Veysi Bahadir[1], Muhsin Kaya[2], Kendal Yalcin[2], Ayhan Kaydu[3]

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Introduction:

The use of transanastomotic internal and external stents in biliary reconstruction during living donor liver transplantation (LDLT) remains controversial. This study aimed to present our postoperative biliary complications using internal transanastomotic catheters in bile duct anastomosis.

Methods:

A retrospective, longitudinal study was conducted on a cohort of LDLT patients treated in our clinic between May 2023 and July 2024.

Results:

The mean age of the patients was 48.34 years [19-67]; 17 (41%) were female, and 24 (59%) were male. Of the grafts, 40 (98.6%) were right lobes, and 1 (2.4%) was a left lobe. The mean follow-up duration was 10.2 months [4-19]. Among the graft bile ducts, 20 (49%) had dual (separate anterior and posterior bile ducts), 13 (31%) were single, 7 (17%) were "glasses-shaped" (fused anterior and posterior bile

ducts), and 1 (2.4%) had triple ducts (anterior, segment 6, segment 7).

All biliary reconstructions used transanastomotic internal stents prepared from feeding catheters of 4-6 French, cut into pieces 4-8 cm long, and sutured with 6/0 Prolene using a continuous posterior and anterior wall technique. A single bile duct received a single 6 French catheter, while dual, glasses-shaped, and triple bile ducts received separate 4-6 French stents for anterior and posterior ducts. Seven (17%) cases utilized the cystic duct for biliary reconstruction, particularly when the anterior-posterior sectors were far apart or in cases of insufficient main hepatic bile ducts for graft anastomosis (Figure 1).

Biloma developed in 2 cases (4.8%) and was resolved with percutaneous drainage on the postoperative 30th and 35th days. There were no late biliary complications that required any intervention.

Discussion:

Biliary complications, such as fistula, biloma, and stricture, are significant causes of morbidity and mortality in LDLT. We need more cases and longer follow-up results, but our results show an exceptionally low biliary complication rate of 4.8%. Therefore, we support using transanastomotic stents as advocated in the literature.

Keywords: Transanastomotic Stent, Liver Transplantation, Biliary Reconstruction, Postoperative Complications

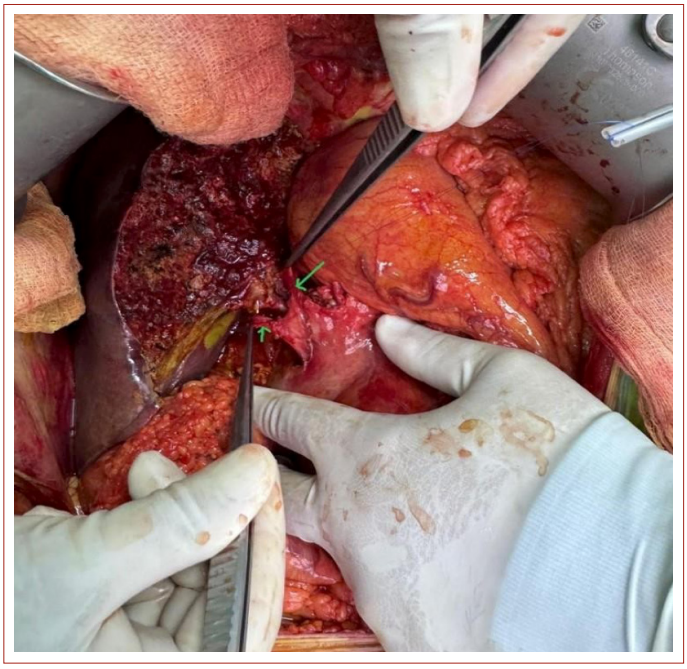

Figure 1. Evaluation of the cystic and common bile duct for anastomosis to the anterior and posterior bile ducts of the right lobe liver grafts.

Short arrow: cystic duct; long arrow: common bile duct.

5. Demanding Liver Transplant Procedures Performed in Our Institution

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Introduction:

Liver transplantation is the only definitive treatment for end-stage liver failure. This study aims to present our experience regarding managing a demanding recipient procedure that was out of the ordinary.

Case Report:

A 53-year-old male patient had been treated for Hepatitis B virus infection for 14 years. He had previously undergone a right hepatectomy in 2020 due to hepatocellular carcinoma. A cadaveric liver transplant was planned for end-stage liver failure.

During recipient hepatectomy, severe perihepatic adhesions from the previous surgery were dissected, and the hepatoduodenal ligament was explored.

The previously ligated right hepatic artery stump was not found, and the left hepatic artery and proximal arterial flow were insufficient. Therefore, a graft was taken from the iliac artery, and reconstruction was performed between the supraceliac aorta and the hepatic artery. Concurrently, portal vein thrombosis was present, and after thrombectomy achieved sufficient flow, portal vein anastomosis was completed.

The surgery lasted 15 hours, with no major complications observed in the early postoperative period. The patient was discharged on postoperative day 30 without issues.

Discussion:

Liver transplantation is a complex surgical procedure, and demanding situations are occasionally encountered. An experienced and multidisciplinary team is crucial for successfully managing such a demanding case.

Keywords: Liver transplantation, iliac artery graft, liver failure, challenging liver transplant cases

6. Superior Mesenteric Artery Syndrome, Hiatal Hernia, and Nutcracker Syndrome Combination: Case Presentation and Review of Literature

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Introduction:

The duodenum and left renal vein lie within the aortomesenteric angle, leading to the definition of two distinct compression syndromes. Due to their similar localization, superior mesenteric artery syn-

drome (SMAS) and nutcracker syndrome (NCS) can occur together. SMAS can cause increased intraluminal pressure, leading to mucosal defects in the stomach and esophagus and hiatal hernia. We aimed to present the first two cases in literature with these three pathologies simultaneously treated in a single surgery.

Methods:

Between May 2021 and September 2024, two patients who underwent simultaneous surgeries for SMAS, NCS, and hiatal hernia from a cohort of four patients treated for SMAS were included in the study. Preoperative symptoms, body mass index (BMI), radiological and endoscopic findings, surgical details, postoperative hospitalizations, and complications were recorded.

Results:

Both patients were male, aged 25 and 40, with BMIs of 16 and 14, respectively. Their symptoms included postprandial pain, vomiting, regurgitation, and weight loss. One patient had a history of two previous surgeries for a hiatal hernia. The patients underwent duodenojejunostomy, Nissen fundoplication, and renal vein transposition. Both patients received anticoagulant therapy postoperatively. One patient had a delayed gastric emptying, requiring an 18-day hospital stay, while the other experienced pneumonia, anastomotic bleeding, and hoarseness, requiring a 25-day stay. No late complications were observed in either patient.

Discussions:

SMAS should be considered in cachectic patients with dyspeptic complaints. Due to similar localization, NCS should be ruled out radiologically in these patients. Endoscopic evaluation of SMAS patients is crucial to exclude mucosal damage and coexisting hiatal hernia. Delayed diagnosis of these syndromes, prioritization of other differential diagnoses, or failure to perform simultaneous surgeries can lead to complications and inappropriate surgeries. Simultaneous surgeries can be safely performed in patients with these combined diagnoses.

Keywords: SMA Syndrome, Nutcracker Syndrome, Hiatal Hernia

7. Multiple Complicated Enteroenteric Intestinal Fistulas Due to Crohn's Disease: A Rare Case Report

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Introduction:

In various studies, fistula formation has been reported in 20-40% of cases of Crohn's disease. Ileosigmoid fistulas are common complications of Crohn's disease with terminal ileum involvement and represent the most frequently observed fistulas between two intestinal segments. Other potential complications of Crohn's disease include enteroenteric, gastroduodenal, duodenocolic, enterovesical, rectovaginal, and perianal fistulas. These fistulas, with challenging treatment protocols, are associated with mortality rates of approximately 6-10%. This report aims to present the diagnosis, surgical treatment, and follow-up process of a case with internal fistulas and complications due to Crohn's disease.

Case Report:

A 54-year-old male patient with a diagnosis of Crohn's disease presented to our clinic with complaints of abdominal pain, diarrhea, and approximately 15 kg weight loss over the past year. He had no previous surgical history. On examination, there was tenderness in the lower abdominal quadrants. Laboratory tests revealed elevated C-reactive protein and white blood cell (WBC) count.

Abdominal ultrasonography (USG) showed wall thickening in the terminal ileum. A computed tomography (CT) enterography scan was performed, revealing wall thickening in the terminal ileum, suspected fistulas between the duodenum and cecum and between the terminal ileum and sigmoid colon, and lymphadenopathies (Figure 1A-D). Colonoscopy showed fistula orifices in the sigmoid colon and terminal ileum. Based on these findings, the patient underwent surgery.

Intraoperatively, millimetric lesions resembling carcinomatosis were observed in the mesentery of the small intestine, along with fistulas between the terminal ileum and sigmoid colon and between the duodenum (2nd-3rd segment) and cecum. Frozen section samples were taken from the millimetric lesions due to suspected carcinomatosis, and the results were benign.

The surgical procedures included ileocecectomy, fistulectomy of the

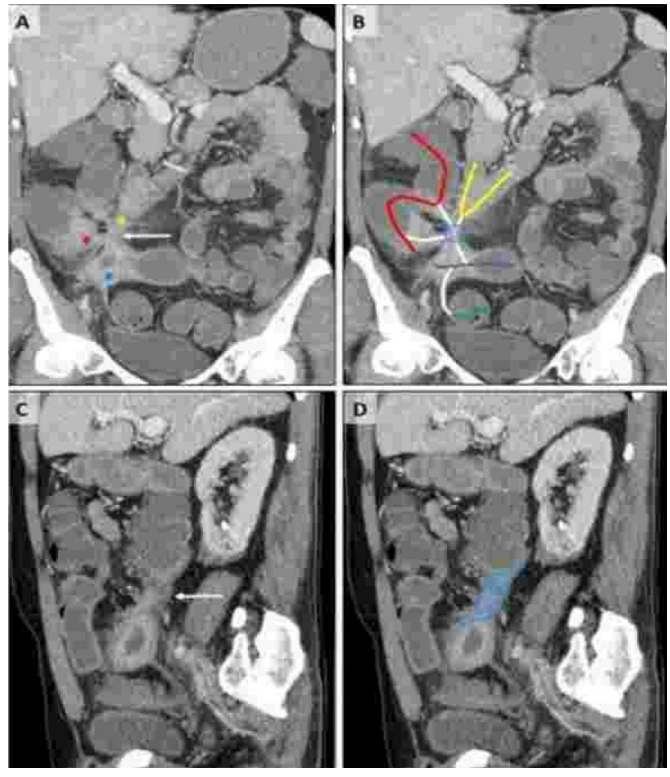

Figure 1. CT Enterography Findings. Coronal (A, B) and sagittal (C, D) MIP (Maximum Intensity Projection) images. Wall thickening and enhanced contrast are observed in the 2nd-3rd part of the duodenum (yellow star), terminal ileum (blue star), and cecum (red star) (A). The lumens of these structures, represented by the same colors, show connections with the sigmoid colon (green line) via tract-like structures (white lines) and fistulization (blue circle) (B). Sagittal images (C, D) reveal a thick-walled fistula tract (C, white arrow) and the fistula tract (D, blue area).

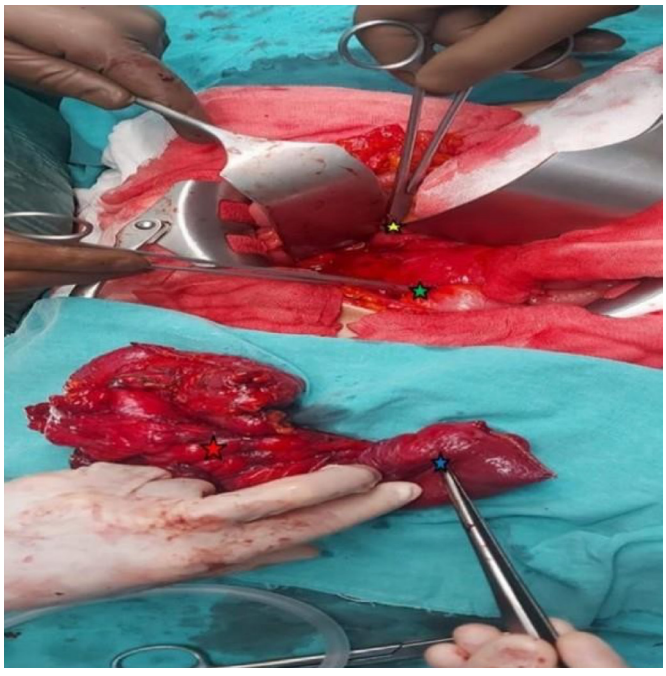

Figure 2. Duodenum's 2nd-3rd parts (yellow star), sigmoid colon (green star), terminal ileum (blue star), and cecum (red star).

duodenal fistula using a linear stapler with duodenal repair, and primary repair of the sigmoid colon fistula site with Vicryl sutures (Figure 2). The patient was monitored in the intensive care unit for one day postoperatively, oral feeding was initiated on day 3, and the patient was discharged on day 8. No postoperative complications were observed. Pathology results confirmed Crohn's disease.

Discussion:

Fistula formation is a serious complication of Crohn's disease. Treating enteroenteric fistulas is complex and requires a multidisciplinary approach. Patients should be referred to specialized centers. Our case serves as an example of diagnosing and surgically managing internal fistula formation due to Crohn's disease.

Keywords: Crohn's Disease, Enteroenteric Fistulas, Surgical Treatment

8. A Rare Complication of Gastric Balloon: Gastric Perforation

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Introduction:

Obesity is a significant health and socioeconomic problem that severely affects quality of life. Gastric balloon applications are increasingly popular in the treatment of obesity. Their reversible nature, lower risks, and fewer complications compared to surgical procedures make them an appropriate option for patients who refuse surgery or cannot undergo it due to comorbidities. Complications of intragastric balloon applications are rarely encountered. Gastric balloons have been used for a long time in the treatment of morbid obesity. Although complications related to the procedure are rare, the most serious reported complications include esophageal or gastric perforations, intestinal obstruction, and death. This study aims to highlight that rare complications can occur, emphasizing the importance of monitoring patients closely.

Case Report:

A 58-year-old female patient weighing 165 kg and measuring 170 cm in height had a swallowable gastric balloon inserted three weeks prior. She was admitted to another center with a three-day history of abdominal pain. After the necessary evaluations, she was referred to our institution with a preliminary diagnosis of gastrointestinal perforation and umbilical hernia.

Upon arrival, the patient exhibited signs of acute abdomen and experienced cardiac arrest in the emergency room. Following five minutes of cardiopulmonary resuscitation (CPR), a pulse was obtained. The patient was taken to surgery urgently with inotropic support. During the surgery, a perforated area measuring 10x6 cm was identified along the lesser curvature of the stomach caused by the balloon (Figures1A and B).

Approximately 7000 cc of food residue was aspirated from the abdominal cavity. The patient experienced another cardiac arrest during the surgery but regained a pulse after CPR. The perforated area in the stomach was resected, and three drains were placed in the abdomen before concluding the operation.

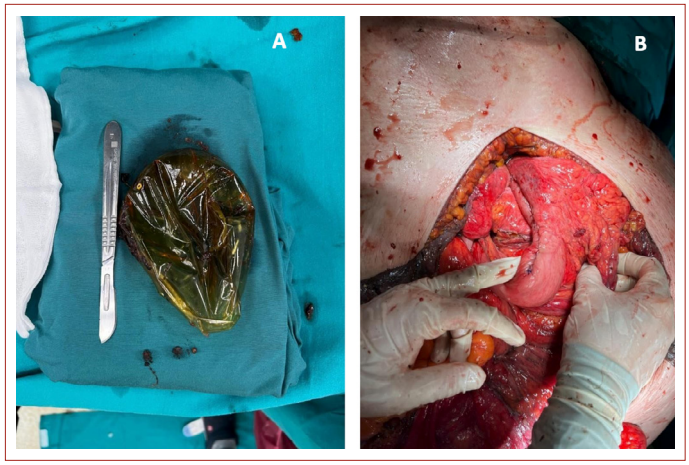

Figure 1. (A) The perforation area on the anterior surface of the stomach. The Injury site is repaired primarily (B) The Gastric band that is removed from the abdominal cavity.

Postoperatively, on the first day, the patient exhibited septic shock and suffered a cardiac arrest. Despite effective CPR, no response was obtained, and the patient was declared as exitus.

Discussion:

Gastric balloons are a less risky alternative to surgical treatments for overweight and obese patients. Close monitoring is recommended for patients at potential risk during the procedure. Early intervention during close monitoring can address possible complications promptly. Close monitoring after balloon insertion in high-risk patients can be life-saving.

Keywords: Gastric balloon, perforation, obesity

9. Laparoscopic Repair of Iatrogenic Colon Perforation During Colonoscopy

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Introduction:

Iatrogenic colon perforations occur in less than 1% of screening colonoscopies but can reach up to 80% in therapeutic procedures. Treatment options include endoscopic repair, conservative approaches, and surgical repair. In recent years, minimally invasive approaches have become prominent in all fields of surgery, including the treatment of this condition. The aim of minimally invasive treatment is to provide positive outcomes such as reduced intra-abdominal trauma, less postoperative pain, and early discharge. In this study, we aim to present a case in our clinic where a successful laparoscopic repair was performed for a colon perforation that occurred during screening colonoscopy.

Case Report:

A 75-year-old patient with a history of coronary artery disease and chronic obstructive pulmonary disease was referred to our clinic for a screening colonoscopy due to anemia. During the initial stage of the colonoscopy, a perforation approximately 0.5 cm in size was observed in the mid-section of the sigmoid colon. Two clips were applied, but as they were deemed insufficient, the procedure was terminated, and the patient was admitted for surgical treatment.

Approximately 3 hours later, the patient underwent surgery. Diagnostic laparoscopy revealed a perforation site of approximately 1 cm on the anterolateral mid-section of the sigmoid colon with no contamination in the abdominal cavity. The perforation site was repaired laparoscopically with a double-layer suture, and the operation was concluded. The patient was discharged uneventfully on postoperative day 6.

Discussion:

Iatrogenic colon perforations during colonoscopy are detected during the procedure in approximately 45-60% of cases, and early intervention provides an advantage for patient recovery. In this case, the perforation site was identified during the procedure. The most common perforation site reported in the literature is the sigmoid colon, which aligns with our case. Similar to the literature, barotrauma was the major contributing factor to perforation in our case. Laparoscopic repair of perforations has been shown to be safe in elderly patients, and our patient was elderly, male, and had comorbid conditions.Iatrogenic colon perforations observed during colonoscopy can be safely and effectively repaired using laparoscopic methods. Identifying the perforation during the procedure is crucial for early intervention and favorable treatment outcomes.

Keywords: Colonoscopy, Iatrogenic colon perforation, Laparoscopic repair

10. Does Splenectomy Affect Mortality and Morbidity in the Postoperative Period of Gastric Cancer?

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Introduction:

To evaluate mortality and morbidity during the postoperative 90-day period in gastric cancer patients undergoing total/subtotal gastrectomy and D2 lymph node dissection with additional splenectomy for various reasons.

Methods:

Gastric cancer patients who underwent total/subtotal gastrectomy, D2 lymph node dissection, and splenectomy between January 2019 and October 2024 were retrospectively analyzed.

Results:

Two (20%) patients were female, with a mean age of 71.1 years. The median operation duration was 4 hours 37 minutes (range: 2 hours 5 minutes to 8 hours 45 minutes). The average hospital stay was 24.7 days. Subtotal/total gastrectomy and D2 lymph node dissection with splenectomy were performed on all patients, with 90% elective surgeries and 10% performed urgently due to tumor perforation.

In the postoperative 90-day period, complications were observed in 30% of patients. Infection sites (wound infection in one patient and intra-abdominal abscesses in two patients) were identified as complications. One patient underwent reoperation due to evisceration. No anastomotic leakage, fistula, bleeding, or mortality was observed in this series of ten patients.

Discussion:

Postoperative complications are a critical concern in clinical practice. Meta-analyses of the literature have shown that morbidity in splenectomy groups is not significantly higher than in spleen-preserving groups. However, some studies suggest that combining splenectomy with gastrectomy increases the risk of morbidity, negatively impacting postoperative recovery. Our results showed that there are no significant early postoperative complications detected in patients undergoing concomitant splenectomy. Subgroup analyses based on different study designs confirmed this finding. While previous studies highlight pancreatitis, anastomotic leakage, abdominal abscess, wound infections, and intestinal obstruction as common postoperative complications following splenectomy, our study demonstrated a low complication rate. No significant difference in mortality was observed between splenectomy and spleen-preserving groups. This retrospective study highlights the need for high-quality, well-

designed, large-scale clinical trials that compare splenectomy with spleen-preserving procedures in a controlled and randomized manner, with short- and long-term assessments.

Keywords: Splenectomy, Gastric cancer, D2 lymph node dissection, Spleen-preserving gastrectomy

11. Pneumatosis Cystoides Intestinalis: Resection of Cysts Without Bowel Resection (First in Literature)

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Introduction:

Pneumatosis Cystoidis Intestinalis (PCI) is a rare condition characterized by gas-filled cysts in the intestinal wall. Some cases present with spontaneous pneumoperitoneum due to ruptured cysts, while others may exhibit acute abdomen-like symptoms due to air retention. The etiology is unclear, with hypotheses suggesting bacterial, mechanical, surgical, or pulmonary origins. Treatments include hyperbaric oxygen, steroids, and surgery. While imaging or incidental findings are cited as diagnostic methods, most cases in studies and reports are diagnosed intraoperatively.

Case Report:

A 27-year-old male patient undergoing amphetamine addiction treatment presented with a five-day history of persistent abdominal pain. Physical examination revealed abdominal distension and tenderness. Imaging showed subdiaphragmatic free air, and CT scans indicated free intra-abdominal air with septation, leading to a delayed perforation diagnosis. Laparotomy revealed gas-filled cysts, averaging 25 cm in length, scattered along the small intestine with 30-40 cm of intact bowel segments in between (Figure 1). No intestinal content or contaminated fluid was found. Resection of four different segments, which would result in short bowel syndrome, was avoided. Instead, the cysts were punctured with electrocautery. Additional cysts in the mesentery were excised, and cysts on the serosal surface were removed using Metzenbaum scissors. Despite resecting approximately 80 cm of bowel, no postoperative leakage or septic conditions occurred. The patient was discharged uneventfully. During a one-year follow-up, the patient experienced one ileus episode, treated non-operatively.

Discussion:

There are many cases where patients with pneumoperitoneum are mistakenly diagnosed with perforation and undergo unnecessary surgical interventions. Although no direct link between amphetamine use and PCI has been established, there may be an indirect association. Amphetamines negatively affect the intestines (vasospasm, ischemic damage, motility disorders, increased intraluminal pressure, and inflammation), potentially contributing to PCI development. The damage caused by amphetamines to intestinal tissue may predispose patients to PCI. The literature primarily includes case reports with no specific treatment protocols for PCI. Surgical interventions generally involve resection. This case represents the first instance of successful cyst resection without bowel resection, demonstrating its effectiveness.

Keywords: Acute abdomen, Amphetamines, Pneumatosis

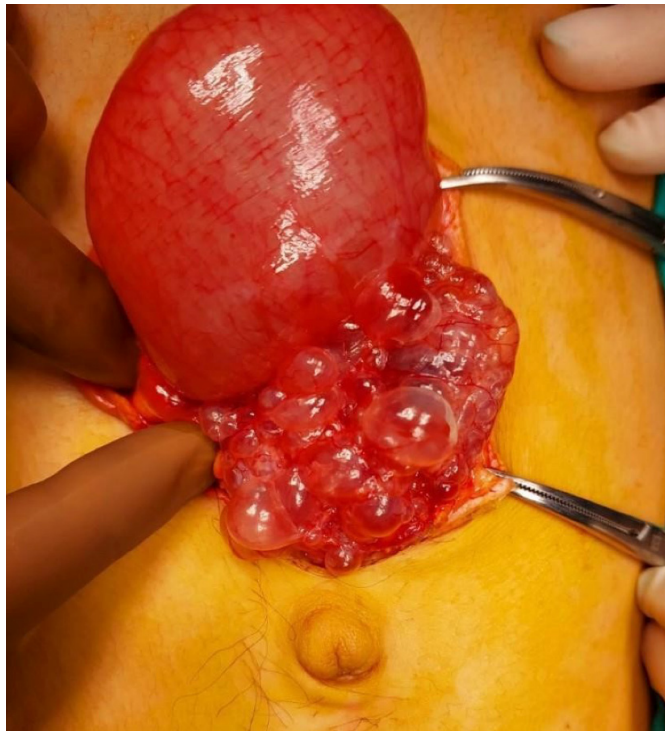

Figure 1. Cystic structures on the intestines during laparotomy.

12. Stricture Due to Igg4-Related Disease Mimicking Perihilar Cholangiocarcinoma: Case Report

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Introduction:

IgG4-related disease can mimic perihilar cholangiocarcinoma by causing short or long segment biliary strictures. The aim of the present study is to evaluate and share our treatment algorithm in patient who was assumed to have cholangiocarcinoma but finally diagnosed as IGG4 related disease.

Case Report:

A 30-year-old male presented to the emergency department with symptoms of obstructive jaundice: total bilirubin 7.6 mg/dL, direct bilirubin 7.2 mg/dL, GGT 276 U/L (<61). CT revealed normal liver vasculature and dilated intrahepatic bile ducts. MRCP identified a stricture dividing the right and left bile ducts, with no mass observed.

Percutaneous transhepatic cholangiography indicated a tumor-like obstruction in the hilar region without findings of sclerosing cholangitis, and bilateral external drainage was performed. Serum IgG4 levels were within normal limits. Despite the patient's age being atypi-

cal, perihilar cholangiocarcinoma could not be ruled out. Extensive hepatobiliary resection was planned, and the right portal vein was embolized percutaneously to enlarge the left lobe.

During surgery, a tumor that did not reach the peritoneum was palpated in the perihilar region. A right hepatectomy, total caudate lobectomy, and Roux-en-Y hepaticojejunostomy were performed. No malignancy was detected in the specimen, which revealed xanthogranulomatous cholecystitis and findings consistent with IgG4-related disease, although not meeting definitive diagnostic criteria.

At 9 months postoperatively, asymptomatic ALT (67 U/L) and GGT (182 U/L) elevations were observed. IgG4 levels remained normal, but MR imaging with gadolinium indicated inflammation-related areas in the liver. The patient was started on 30 mg/day prednisolone, and biochemical findings normalized within a month. Steroid doses were gradually tapered, and azathioprine was added. No clinical or biochemical issues were observed during six months of follow-up.

Discussion:

Recurrence can occur in the remaining liver in cases diagnosed with IgG4-related disease after hepatobiliary resection for suspected perihilar cholangiocarcinoma. Lifelong follow-up is necessary, and immunosuppressive therapy should be initiated if biochemical or radiological findings emerge.

Keywords: Perihilar cholangiocarcinoma, IgG4-related cholangitis, Klatskin tumor, sclerosing cholangitis, xanthogranulomatous cholecystitis

13. Cytoreductive Surgery and Hyperthermic Intraperitoneal Chemotherapy Case in a Liver Transplant Recipient

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Introduction:

Cytoreductive surgery combined with intraperitoneal chemotherapy (SRC+HYPEC) is an effective treatment modality for peritoneal malignancies, including pseudomyxoma peritonei, malignant peritoneal mesothelioma, colon cancer, gastric cancer, and ovarian carcinoma. SRC+HYPEC has significantly improved overall survival rates in colorectal cancer with peritoneal dissemination, achieving a 5-year survival rate of up to 45% with complete cytoreduction. De novo malignancies can also develop following liver transplantation, where SRC+HYPEC can be safely performed.

Case Report:

A 54-year-old male patient who underwent liver transplantation for ethanol-induced liver cirrhosis developed right-sided colon cancer with peritoneal metastasis four years post-transplant. The patient received six cycles of FOLFOX chemotherapy and subsequently underwent SRC+HYPEC (Figures 1A and B). The postoperative period was uneventful.

Discussion:

SRC+HYPEC is now considered the gold standard treatment for colorectal cancer with peritoneal dissemination. Key prognostic indicators include a reduction in the peritoneal carcinomatosis index and

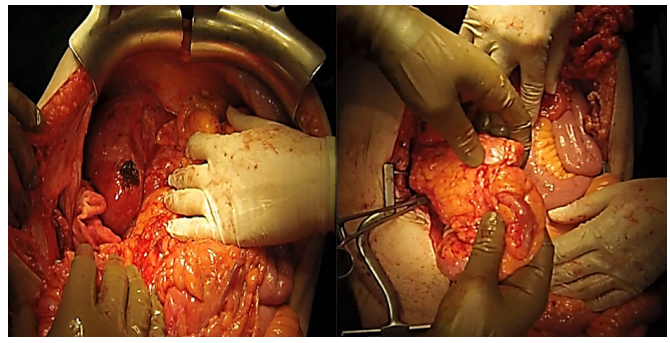

Figure 1. (A) The de novo right-sided colon cancer in our transplant recipient (B)The peritoneal dissemination of the tumor.

achieving complete cytoreduction. Colorectal cancer is a prevalent de novo malignancy following liver transplantation, underscoring the importance of pre- and postoperative screening programs. The success of SRC+HYPEC is influenced by factors such as the peritoneal carcinomatosis index, operative duration, and blood loss, all of which necessitate careful patient selection for the procedure.

Keywords: Liver transplantation, Cytoreductive surgery, HIPEC

14. Combined Complications After Whipple Procedure: Intraluminal Bleeding And Pancreatic Fistulas

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Introduction:

Despite improved surgical experience and patient management, mortality rates are decreasing, but morbidity remains a concern even in high-volume centers. Postoperative complications are now managed with multidisciplinary approaches. Rare complications that can lead to mortality may occur in combination. Our goal is to present the combined complications we encountered and their management in this case.

Case Report:

A 58-year-old male underwent pancreaticoduodenectomy due to a mass in the pancreatic head. On postoperative day 3, the drain amylase level was consistent with a pancreatic fistula. On day 5, the patient developed hematemesis during intensive care follow-up. Gastroscopy revealed hemorrhage at the gastrojejunostomy anastomosis, which was treated with sclerotherapy.

On day 8, CT imaging showed dilated small intestines, gastrojejunostomy, and jejunojejunostomy with proximal dilation. Additionally, bile began to drain from abdominal drains. During surgery, disruption of the jejunojejunostomy anastomosis was noted and repaired. Separation of 30% of the pancreatojejunostomy and hepatojejunostomy anastomoses was observed. Pancreatojejunostomy was reinforced, the omega loop was dismantled, and a Roux-en-Y anastomosis was performed. The patient was discharged on postoperative day 45.

Discussion:

Complications after pancreaticoduodenectomy, especially postoperative pancreatic fistulas, are serious conditions that can lead to mortality. Combined complications complicate postoperative management and require a multidisciplinary approach. Effective management of such cases necessitates collaboration between interventional radiology, gastroenterology, and general surgery teams. Complicated complications after pancreaticoduodenectomy require experienced surgical teams and multidisciplinary approaches for effective management. This case demonstrates the importance of a multidisciplinary approach in handling combined complications.

Keywords: Whipple, Pancreatic fistula, Pancreaticoduodenectomy

15. Hepatoid Adenocarcinoma Metastasis of the Stomach Mimicking Perforation: Case Report

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Introduction:

Hepatoid adenocarcinoma is a rare neoplasm occurring in organs or tissues other than the liver, characterized by hepatocyte-like differentiation and morphology resembling hepatocellular carcinoma. The stomach is one of the most common sites for hepatoid adenocarcinomas. This report presents a case diagnosed with bile produced from intra-abdominal metastatic areas.

Case Presentation:

A 73-year-old female patient was evaluated for abdominal pain during intensive care unit admission, and free pus was detected in the abdomen. Abdominal ultrasonography revealed a non-cirrhotic liver parenchyma, and viral serology was negative. WBC: 11.6; CRP: 174 mg/L. Radiological imaging showed no perforation.

USG-guided paracentesis revealed bile content, leading to a suspicion of acute abdomen, and the patient underwent diagnostic laparotomy. No perforation was detected in intra-abdominal organs during exploration. Excisional biopsy samples were taken from several omental implants, and cholecystectomy was performed (Figure 1A). Histopathological evaluation confirmed hepatoid adenocarcinoma metastasis of gastric origin (Figure 1B).

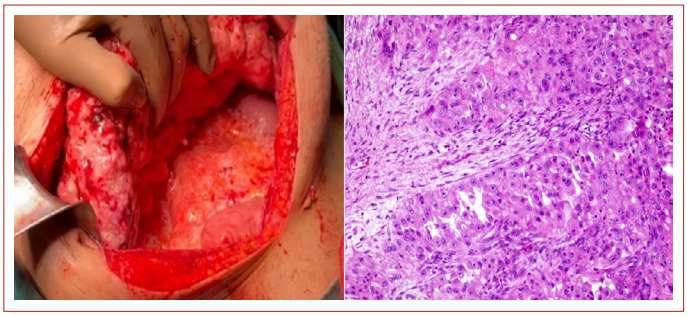

Figure 1. (A) Intraoperative view of the patient (B)Microphotograph of polygonal-shaped cell islands with prominent eosinophilic cytoplasm belonging to hepatoid adenocarcinoma (Hematoxylin & Eosin stain, X200).

Discussion:

Hepatoid adenocarcinoma of the stomach is typically seen in advanced age and exhibits an aggressive clinical course with poor prognosis. Diagnosis is usually made in advanced stages due to the lack of specific clinical symptoms and imaging findings. In this case, bile formed with hepatocyte-like differentiation facilitated the decision for emergency surgery. There is no standard treatment for this neoplasm, which can occur in various organs. Further studies are needed to develop better diagnostic and treatment plans.

Keywords: Hepatoid adenocarcinoma metastasis, Perforation, Surgery

16. Our Experience With Malignant Gallbladder Tumors

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Introduction:

Gallbladder malignancy is the most common and aggressive malignancy of the biliary tract and ranks as the 5th most frequent tumor in the gastrointestinal system. It is a rare disease. Gallbladder cancer remains a highly mortal disease, as only a small portion of patients are diagnosed at a stage amenable to surgical resection. It may present as direct invasion into adjacent organs, local lymph node metastasis, diffuse metastatic disease, or incidentally during cholecystectomy.

This study aims to present patients diagnosed with malignant gallbladder tumors following surgery at our center.

Methods:

A retrospective evaluation was conducted of 11 patients diagnosed with malignant gallbladder tumors among 6200 cholecystectomy cases performed for various indications in our clinic between September 2019 and September 2024.

Results:

The mean age of the 11 patients with malignant gallbladder tumors was 70 (range: 48–82 years); 2 (18%) were male, and 9 (82%) were female. Malignancy was incidentally detected post-laparoscopic cholecystectomy in 6 patients. Pathological reports of laparoscopic cholecystectomy cases indicated 4 as pT2, 1 as pT1, and 1 as pT3. Three patients subsequently underwent definitive surgery.

Post-definitive surgery pathology revealed no malignancy, and the average hospital stay was 13 days. Postoperative complications included wound infection in 1 patient and ileus in another, the latter requiring surgery for adhesiolysis. One patient was urgently operated on due to gallbladder perforation, with pathology reported as pT3N1.

Four patients suspected of malignancy underwent open surgery. Among these, 1 was reported as pT2, 1 as pT3N2MX, 1 as pT4, and 1 as pT3N1. The patient reported as pT3N1 died on postoperative day 63 due to comorbid conditions. The average hospital stay for open surgery cases was 22 days. In total, one patient followed in the hospital died.

Discussion:

Gallbladder malignancy is a rare tumor and is generally diagnosed at advanced stages. Female gender and advanced age are demographic risk factors for gallbladder cancer. Adenocarcinoma constitutes the majority of gallbladder cancers. Surgery remains the only curative treatment for gallbladder cancer. Our study contains results consistent with the literature.

Keywords: Gallbladder, Gallbladder tumor, Adenocarcinoma, Cholecystectomy

17. Hepatocellular Carcinoma with Tumor Thrombus Extending Into the Right Atrium: Case Report

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Introduction:

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer-related deaths. Risk factors include hepatitis B and C virus infections and other cirrhosis-inducing factors, although it may sometimes occur as a primary tumor. Intrahepatic vascular invasion and tumor thrombus are common in HCC. However, the extension of tumor thrombus into the inferior vena cava (IVC) and subsequently into the right atrium is rare. This case aims to present successful surgical management of an HCC case complicated by tumor thrombus extending into the IVC and right atrium.

Case Presentation:

A 72-year-old male patient presented with a mass in the right lobe of the liver. Triphasic dynamic computed tomography revealed a tumor measuring 140x130x115 mm in the right lobe of the liver. The patient's serum alpha-fetoprotein (AFP) level was 19.6, and ELISA blood values were normal. Abdominal computerized tomography revealed that the tumor thrombus was observed to extend through the inferior vena cava (IVC) into the right atrium (Figure 1). After completing preoperative preparations, the patient underwent thromboendovenectomy and right hepatectomy (Figure 2) . Postoperative intensive care and ward follow-ups were uneventful, and the patient was discharged on postoperative day 5. At the one-month follow-up, the AFP level was 3.8, and no residual tumor was detected on CT imaging. The patient remains stable (Figure 3).

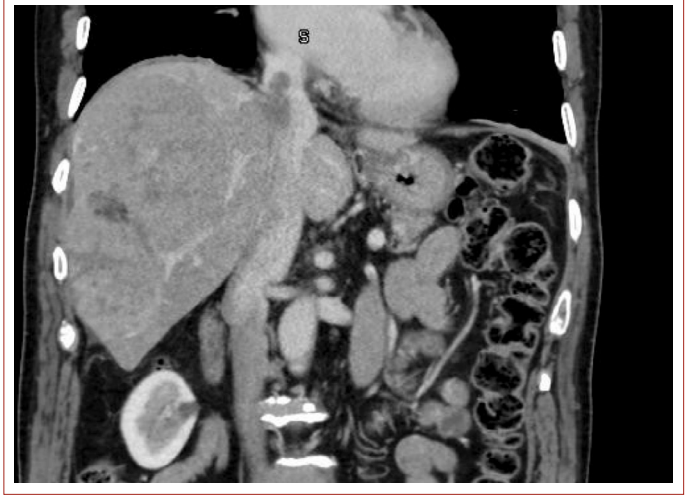

Figure 1. CT image showing the tumor in the right lobe of the liver and the thrombus extending from the inferior vena cava (IVC) into the right atrium.

Discussion:

In cases where hepatic tumors are resectable, complete removal of the primary tumor and associated thrombotic mass is possible with surgical intervention. However, surgical indications must be evaluated meticulously.

Keywords: Inferior vena cava, Right atrium, Hepatocellular carcinoma

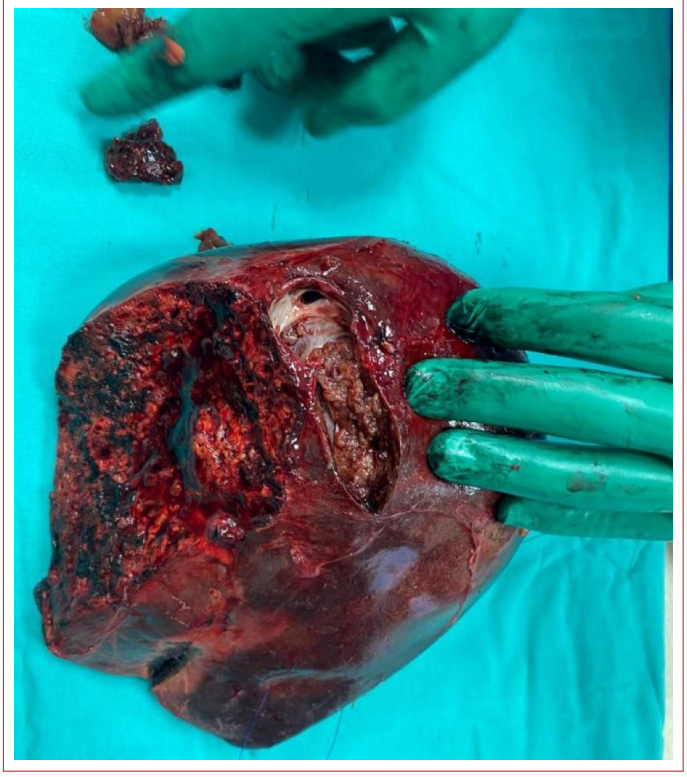

Figure 2. Images showing the tumor and thrombus during and after the surgery.

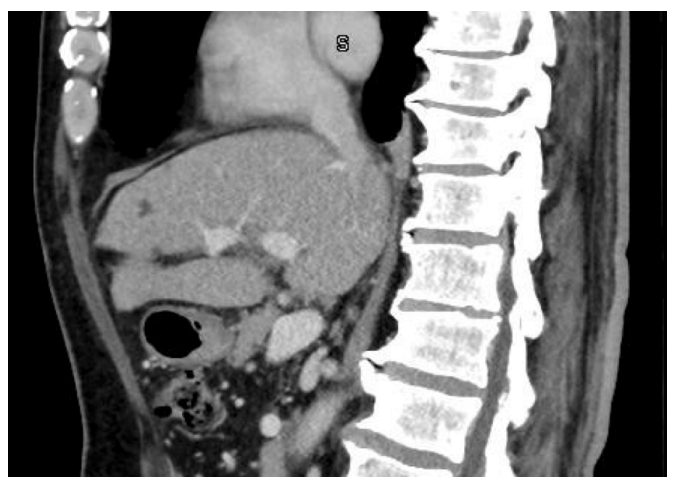

Figure 3. The CT images on the postoperative 3rd month of the patient.

18. Management of a Trapped Basket in the Common Bile Duct: Challenging Situation in Ercp

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Introduction:

Endoscopic retrograde cholangiopancreatography (ERCP) is a widely used method for managing bile duct stones. While common complications include pancreatitis, bleeding, and cholangitis, rare occurrences such as a Dormia® basket becoming trapped can also be observed. This study presents a case where a Dormia® basket trapped in the common bile duct during ERCP was removed through open surgery.

Case Report:

An 80-year-old male patient with coronary artery disease and hypothyroidism, who had experienced multiple cholecystitis attacks in the past, presented to the emergency department with complaints of jaundice, fever, abdominal pain, nausea, and vomiting. Physical examination revealed an icteric appearance and a positive Murphy's sign. Laboratory tests showed elevated bilirubin levels. Imaging identified an 11 mm gallstone in the gallbladder and a 7 mm stone in the common bile duct (choledocholithiasis).

During ERCP, stone extraction was attempted using a balloon basket; however, the basket catheter became stuck in the bile duct. Various balloons were used to attempt papilla dilation, but the basket could not be removed. Consequently, emergency surgery was planned.

During open surgery, the common bile duct was explored, and the basket and stones were successfully removed (Figures 1A and B). The patient was discharged on postoperative day 13 with a cystic duct cannula.

Discussion:

Common ERCP complications include hyperamylasemia, pancreatitis, and perforation. Basket entrapment occurs in 0.8-5.9% of cases, typically caused by large, hard stones. Various procedures have been reported for removing trapped stones and Dormia® baskets, including sphincterotomy enlargement, extracorporeal shockwave lithotripsy (ESWL), mechanical, electrohydraulic, or laser lithotripsy, dissolving agents, and percutaneous methods. When these methods fail, surgical intervention is usually necessary. ERCP is an effective method for managing biliary issues; however, sufficient expertise and resources are required to handle rare complications. If other methods fail, surgical intervention should be considered as a second-line option.

Keywords: Dormia basket, ERCP, choledocholithiasis

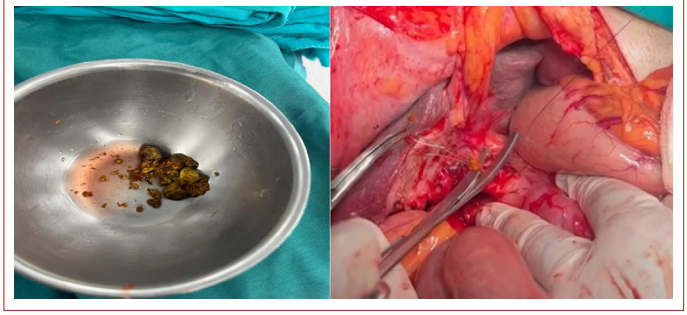

Figure 1. (A) Trapped basket in the common bile duct (B) The extracted stones.

19. Combined Endoscopic and Surgical Approach in the Treatment of Hepatolithiasis

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Introduction:

Hepaticojejunostomy (HJ) is a commonly used surgical approach in the treatment of severe biliary pathologies such as bile duct injuries. Over time, obstruction or stone formation can occur at the HJ anastomosis site. This case report aims to present a successful revision surgery performed using a combined endoscopic and surgical approach in a patient who previously underwent HJ due to bile duct injury and presented with obstructive jaundice due to bilateral hepatolithiasis.

Case Report:

A 50-year-old male patient developed a bile duct injury during a cholecystectomy in 2014, which necessitated an HJ. The patient presented with complaints of obstructive jaundice, right upper quadrant pain, jaundice, and loss of appetite. Laboratory tests revealed total bilirubin/direct bilirubin levels of 11.8 mg/dL / 8.5 mg/dL, alkaline phosphatase of 247, GGT of 109, and elevated AST and ALT.

Abdominal CT and MR imaging showed dilated intrahepatic bile ducts and bilateral hepatolithiasis. Additionally, dilated venous collateral vascular structures were observed in the splenic hilum and pericardial area of the stomach, suggesting secondary biliary cirrhosis. Initially, a percutaneous transhepatic catheter was placed. Since interventional radiology determined that the stones could not be removed percutaneously, surgery was planned.

The existing hepaticojejunostomy anastomosis and enteroenterostomy were evaluated. An enterotomy was performed in the afferent limb of the enteroenterostomy, allowing endoscopic access. The hepaticojejunostomy anastomosis was reached endoscopically, and the percutaneous transhepatic cholangiography (PTC) catheters were visualized.

The status of the stones was assessed via catheters, and intrahepatic bile duct stones were confirmed by cholangiography. Balloon dilation was performed to widen the hepaticojejunostomy anastomosis, removing some stones and sludge. However, due to incomplete visualization of the bile duct and insufficient anastomosis, it was decided to revise the HJ.

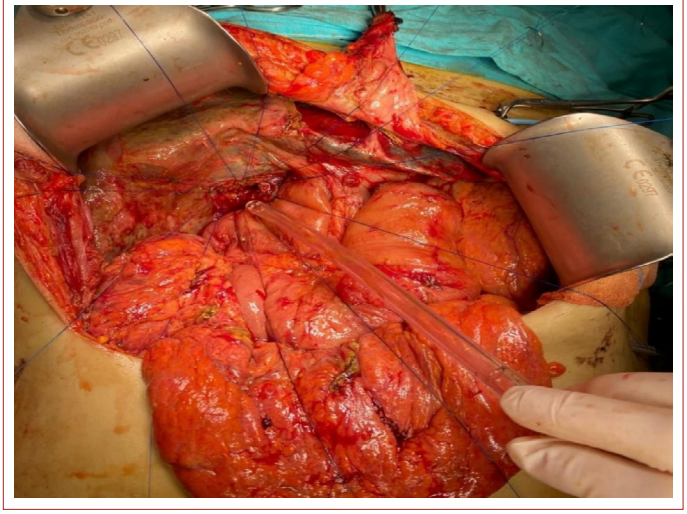

Figure 1. Dismantling of the hepaticojejunostomy.

The hepaticojejunostomy was dismantled, and anterior and posterior sector ducts of the liver's left lobe were individually cleared of stones (Figure 1). The hepaticojejunostomy was reconstructed using the Roux-en-Y method. PTC catheters were placed into the small intestine. The patient was discharged in good health on postoperative day 18.

Discussion:

The diagnosis, complications, and definitive surgical treatment of hepatolithiasis require a challenging and multidisciplinary approach. With experienced centers and a multidisciplinary approach, definitive treatment can be performed safely and effectively.

Keywords: Hepatolithiasis, Hepaticojejunostomy, Biliary stricture

20. Endoscopic Approach to Pancreatic Pseudocyst: Single-Center Experience

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Introduction:

Pancreatic pseudocyst is the most common cystic lesion of the pancreas, usually developing on a background of pancreatitis. This study aims to present cases of pancreatic pseudocyst treated with endoscopic cystogastrostomy.

Materials and Methods:

Patients who underwent endoscopic procedures for pancreatic pseudocysts between January 2021 and June 2024 were retrospectively evaluated.

Results:

Of the patients, 10 (66.7%) were female, 5 (33.3%) were male, with a mean age of 48.2 years. The etiology of pseudocysts were biliary pancreatitis in 13 (86.7%) patients and postpartum pancreatitis in 2 (13.3%) patients. Endoscopic stents were placed in 9 (60%) patients via cystogastrostomy (Figure 1). Four (26.7%) patients underwent laparotomy due to unsuccessful endoscopic procedures, and 2 (13.3%) patients underwent direct surgical cystogastrostomy. The average time between pancreatitis and invasive intervention was 115 days. Repeat

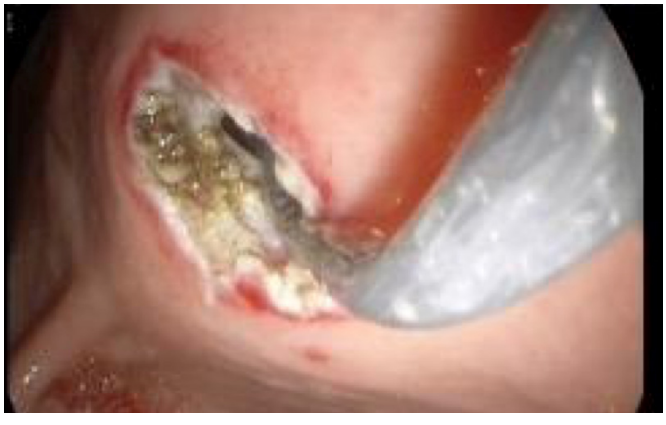

Figure 1. Endoscopic cystogastrostomy.

Table 1. The clinical and demographic characteristics of the patients

Age	Gender	Indication	Percutanous Drainage	Endoscopic Intervention	Cystogastrostomy	Duration of Hospitalization	Recurrent	Stent Placement	Follow Up (Mo)	Complications	Classifications
35	F	Postpartum biliary pancreatitis	-	successful	Endoscopic stent	45	positive	-	7	-	4
66	F	Biliary pancreatitis	-	-	laparatomy	60	-	-	7	Surgical site infection	4
33	F	Postpartum biliary pancreatitis	successful	successful	Endoscopic stent	90	positive	positive	6	-	4
24	F	biliary pancreatitis	-	successful	Endoscopic stent	60	positive	-	5	-	4
50	M	Biliary pancreatitis	-	Failure	laparatomy	150	-	-	8	-	4
69	M	Biliary pancreatitis	-	Failure	laparatomy	45	positive	-	10	Surgical site infection	4
54	M	Biliary pancreatitis	-	Failure	laparatomy	360	-	-	5	-	4
58	F	Biliary pancreatitis	successful	-	laparatomy	45	-	-	12	Intraabdominal abscess	4
61	F	Biliary pancreatitis	-	Failure	Endoscopic stent	180	positive	positive	3	-	4
46	F	Biliary pancreatitis	-	successful	Endoscopic stent	50	-	-	3	-	4
53	F	biliary pancreatitis	-	successful	Endoscopic stent	100	-	-	3	-	
56	M	Biliary pancreatitis	-	successful	Endoscopic stent	60	positive	positive	3	-	4
34	M	Biliary pancreatitis	successful	successful	Endoscopic stent	40	-	-	3	-	4
34	F	Biliary pancreatitis	-	successful	Endoscopic stent	45	positive	positive	5	-	4
50	F	Biliary pancreatitis	-	Failure	Laparotomy	70	-	-	5	Pancreatic fistula	4

procedures were required in 6 of the patients with stents and 1 patient who underwent laparotomy. Stent replacements were performed in 4 patients. According to the pan-pseudocyst classification, all patients were type 4. During an average follow-up of 7 months, 2 patients developed wound infections, 1 patient had an intra-abdominal abscess, and 1 patient experienced a pancreatic fistula (Table 1).

Discussion:

Minimally invasive endoscopic drainage with stent placement for cystogastrostomy can be considered the first-line treatment for pancreatic pseudocysts.

Keywords: Pseudocyst, Endoscopic cystogastrostomy, Pancreatitis

21. Effectiveness of Biomarkers in Predicting Recurrence in Intra-Abdominal Sarcomas

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Introduction:

The aim of this study is to investigate whether biochemical markers such as hemoglobin, albumin, lymphocyte, and platelet (HALP) scores, lymphocyte-C-reactive protein ratio (LCR), and CRP/Albumin ratio can predict survival and recurrence in patients with intra-abdominal sarcomas.

Methods:

This retrospective study included adult patients (aged 18 and older) who underwent surgery for intra-abdominal sarcoma and experienced recurrence (n=23) between January 2014 and March 2024. Patients were divided into two groups: those with recurrence (n=13) and those without recurrence (n=10). Preoperative biochemical data were used to calculate HALP, CRP/Albumin scores, and LCR ratios, and their predictive ability for early pathological findings was analyzed.

Results:

Among the non-recurrence group, 5 patients (21.7%) were male, while 6 patients (34.8%) in the recurrence group were male. The mean age of patients with recurrence was 55.44±15.27 years (range: 23-70), with an average survival of 22.44±20.68 months (range: 0-60). The mean age of patients without recurrence was 67.13±14.14 years (range: 44-79), with an average survival of 26.75±28.78 months (range: 6-84).The cutoff values for intra-abdominal sarcomas were determined as 0.68 for CRP/Albumin, 29.42 for HALP, and 0.42 for LCR. Patients with low HALP scores had significantly shorter disease-free survival (18.83 months) compared to those with high HALP scores (24.55 months, $p<0.01$). Similarly, patients with low LCR scores had significantly shorter disease-free survival (22.50 months) than those with high LCR scores (25.05 months, $p<0.01$). Patients with low CRP/Albumin values had significantly longer disease-free survival (18.63 months) compared to those with high CRP/Albumin values (29.67 months, $p<0.01$). A strong correlation was found between HALP, LCR, CRP/Albumin scores, and recurrence ($p<0.05$).

Discussion:

Low HALP, low LCR, and low CRP/Albumin scores were identified as significant independent prognostic factors for predicting postoperative overall survival and recurrence in intra-abdominal sarcoma patients.

Keywords: Intra-abdominal sarcoma cancer, biomarkers, prognosis, recurrence, survival

22. Subtotal Cholecystectomies in Difficult Gallbladder Surgeries

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Introduction:

Laparoscopic cholecystectomy is the gold standard for treating acute cholecystitis and cholelithiasis. However, in some cases, subtotal cholecystectomy may be preferred to avoid bile duct injuries.

Methods:

Ten patients who underwent subtotal cholecystectomy in our clinic between 2023 and 2024 were analyzed.

Results:

Seven patients (70%) were female, with an average age of 48 years (range: 25-78). The average hospital stay was 3.8 days (range: 1-10). All patients had acute calculous cholecystitis, and 8 (80%) had choledocholithiasis.

Preoperative ERCP (Endoscopic Retrograde Cholangiopancreatography) was performed in 7 (70%) patients, while 1 patient (10%) underwent PTK (Percutaneous Transhepatic Cholangiography). Laparoscopic surgery was completed in 9 (90%) patients, while 1 patient (10%) required conversion to open surgery. In subtotal cholecystectomy cases, the residual gallbladder stump was closed using staples in 5 (50%) patients and sutures in 5 (50%). No mortality was observed. Morbidity included wound infections in 2 (20%) patients, and postoperative atelectasis and pneumonia in 3 (30%) patients (Table 1). No bile fistula occurred.

Discussion:

The primary goal in laparoscopic cholecystectomy is safety. Subtotal cholecystectomy is a viable option for ensuring safe cholecystectomy, particularly in patients with acute episodes, a history of choledocholithiasis, or prior ERCP.

Keywords: Subtotal cholecystectomy, acute cholecystitis, ERCP

Table 1. Clinical Characteristics of the patients with subtotal cholecystectomy

Patient no.	Age	Gender	Stump Closure Methodology	Duration of Hospitalization	Preoperative interventions	Cholecystectomy type
1	63	Male	Stapler	8	-	Laparoscopic
2	77	Female	Suture	3	PTC insertion + ERCP	Laparoscopic to open conversion
3	61	Female	Suture	10	ERCP	Laparoscopic
4	37	Female	Suture	1	Cystogastrostomy	Laparoscopic
5	35	Female	Suture	1	ERCP	Laparoscopic
6	35	Male	Stapler	2	ERCP+ Cystogastrostomy	Laparoscopic
7	60	Female	Suture	3	ERCP	Laparoscopic
8	70	Male	Stapler	3	-	Laparoscopic
9	25	Female	Stapler	2	-	Laparoscopic
10	78	Female	Stapler	5	ERCP	Laparoscopic

PTC: Percutaneous transhepatic cholangio-pancreatico-ductograhy assisted catheter
ERCP: Endoscopic retrograde pancreatico-ductograhy.