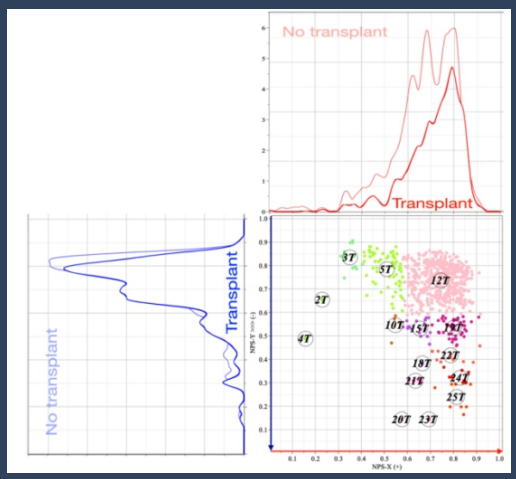
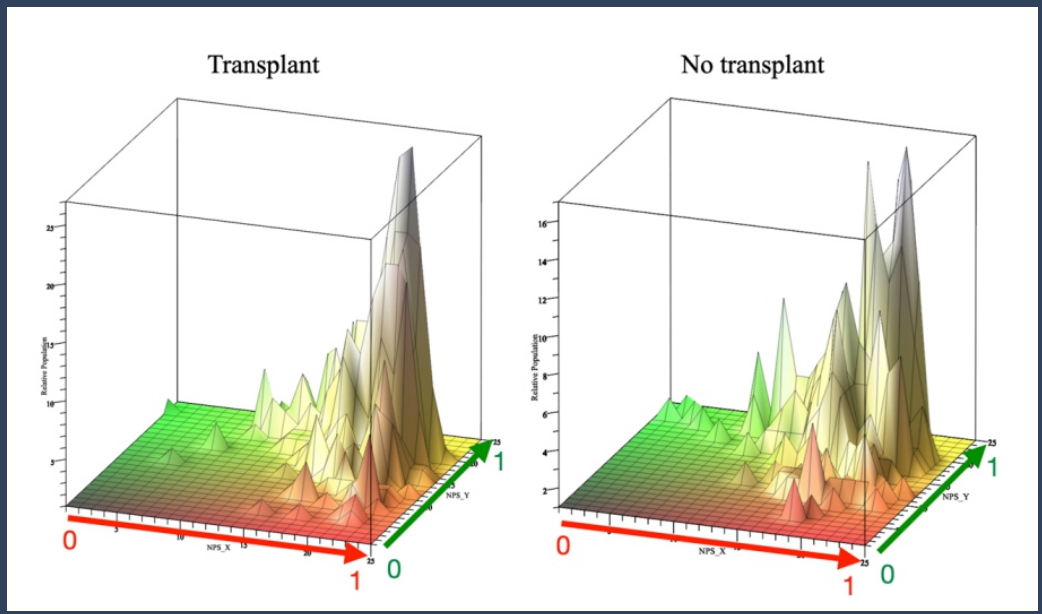
The Official Journal of the Inonu Liver Transplantation Institute

Journal of Inonu Liver Transplantation Institute



A Network Phenotyping Strategy approach in a Turkish HCC dataset from Inonu University and comparison of patients selected for transplant and those who were not



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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
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JOURNAL OF INONU LIVER TRANSPLANTATION INSTITUTE

JILTI

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.

Ethics and Policies

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Each individual listed as an author should fulfill the authorship criteria recommended by the International Committee of Medical Journal Editors (ICMJE). The ICMJE recommends that authorship should 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 provided a contribution but do not meet all four criteria should be recognized separately on the title page and in the Acknowledgements section at the conclusion of the manuscript.
The Journal of Inonu Liver Transplantation Institute requires that corresponding authors submit a signed and scanned version of the authorship contribution form available for download through during the initial submission process in order to appropriately indicate and observe authorship rights and to prevent ghost or honorary authorship. Please note that the list of authors on the final manuscript will be presented in the order provided on this form. 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.

Ethics Policy

The Editorial Board of the Journal of Inonu Liver Transplantation Institute and the Publisher adheres to the principles of the International Council of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME), the Council of Science Editors (CSE), the Committee on Publication Ethics (COPE), the US National Library of Medicine (NLM), the World Medical Association (WMA) and the European Association of Science Editors (EASE).
In accordance with the journal's policy, an approval of research protocols by an ethics committee in accordance with international agreements "WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects (last updated: October 2013, Fortaleza, Brazil)" , "Guide for the care and use of laboratory animals (8th edition, 2011)" and/or "International Guiding Principles for Biomedical Research Involving Animals (2012)" is required for all research studies. If the submitted manuscript does not include ethics committee approval, it will be reviewed according to COPE's guideline (Guidance for Editors: Research, Audit and Service Evaluations). If the study should have ethical approval, authors will be asked to provide ethical approval in order to proceed the review process. If they cannot provide ethical approval, their manuscript will be rejected and also their institutions and when needed, the related bodies in their country will be informed that such studies must have ethics committee approval. If they provide approval, review of the manuscript will continue.
For articles concerning experimental research on humans, a statement should be included that shows informed consent of patients and volunteers was obtained following a detailed explanation of the procedures that they may undergo. The journal may request a copy of the Ethics Committee Approval received from the relevant authority. Informed consent must also be obtained for case reports and clinical images.
Studies using human or animal subjects should be approved by the appropriate institutional and local Ministry of Health ethics committees. Ethics approval of research protocols in accordance with international agreements is required for experimental, clinical, and drug studies, as well as for some case reports. Ethics committee reports or an equivalent official document may be requested from the authors. For manuscripts involving experimental research on humans, a statement should be included that shows that written, informed consent of patients and volunteers was obtained. For studies carried out on animals, the measures taken to prevent pain and suffering of the animals should be stated clearly. A statement regarding patient consent, and the name of the ethics committee, the ethics committee approval date, and number should be stated in the Materials and Methods section of the manuscript. It is the authors' responsibility to carefully protect patients' anonymity.

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All submissions are screened using similarity detection software at least two times: on submission and after completing revisions. In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, or data falsification/fabrication, the editorial board will follow and act in accordance with COPE guidelines. Plagiarism, including self-plagiarism, that is identified at any stage will result in rejection of the manuscript.

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The Journal of Inonu Liver Transplantation Institute supports the Budapest Open Access Initiative statement of principles that promotes free access to research literature. The declaration defines open access to academic literature as free availability on the internet, permitting users to read, record, copy, print, search, or link to the full text, examine them for indexing, use them as data for software or other lawful purposes without financial, legal, or technical barriers. Information sharing represents a public good, and is essential to the advancement of science. Therefore, articles published in this journal are available for use by researchers and other readers without permission from the author or the publisher provided that the author and the original source are cited. The articles in the Journal of Inonu Liver Transplantation Institute are accessible through search engines, websites, blogs, and other digital platforms. Additional details on the Budapest Open Access Initiative and their guidelines are available at https://www.budapestopenaccessinitiative.org/

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Only those manuscripts approved by its every individual author and that were not published before in or sent to another journal, are accepted for evaluation.

Submitted manuscripts that pass preliminary control are scanned for plagiarism using iThenticate software. After plagiarism check, the eligible ones are evaluated by Editor-in-Chief for their originality, methodology, the importance of the subject covered and compliance with the journal scope. Editor-in-Chief evaluates manuscripts for their scientific content without regard to ethnic origin, gender, sexual orientation, citizenship, religious belief or political philosophy of the authors and ensures a fair double-blind peer review of the selected manuscripts.

The selected manuscripts are sent to at least two national/international referees for evaluation and publication decision is given by Editor-in-Chief upon modification by the authors in accordance with the referees' claims.

Editor-in-Chief does not allow any conflicts of interest between the authors, editors and reviewers and is responsible for final decision for publication of the manuscripts in the Journal.

Reviewers' judgments must be objective. Reviewers' comments on the following aspects are expected while conducting the review.

- Does the manuscript contain new and significant information?
- Does the abstract clearly and accurately describe the content of the manuscript?
- Is the problem significant and concisely stated?
- Are the methods described comprehensively?
- Are the interpretations and consclusions justified by the results?
- Are adequate references made to other Works in the field?
- Is the language acceptable?

Reviewers must ensure that all the information related to submitted manuscripts is kept as confidential and must report to the editor if they are aware of copyright infringement and plagiarism on the author's side.

A reviewer who feels unqualified to review the topic of a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

The editor informs the reviewers that the manuscripts are confidential information and that this is a privileged interaction. The reviewers and editorial board cannot discuss the manuscripts with other persons. The anonymity of the referees is important.

Archiving Policy

The content published by the Journal of Inonu Liver Transplantation Institute is electronically preserved by using Internat Archieve.

Fee Waiver Policy

There is no fee waiver.

Funding Sources Policy

All authors are required to declare what support they received to carry out their research. Declaring funding sources acknowledges funders' contributions, fulfills funding requirements, and promotes greater transparency in the research process.

Each author must individually declare all sources of funding received for the research submitted to the journal. This information includes the name of granting agencies, grant numbers, and a description of each funder's role. If the funder has played no role in the research, this must be stated as well.

Authors are not required to provide the complete list of every single grant that supports them if the grant is not related to the research published.

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The Journal of Inonu Liver Transplantation Institute assesses no submission fees, publication fees, or page charges.

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If the editors or publisher learn from a third party that a published work contains a material error or inaccuracy, the authors must promptly correct or retract the article or provide the journal editors with evidence of the accuracy of the article.

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The Journal of Inonu Liver Transplantation Institute is committed to providing high quality articles and uphold the publication ethics to advance the intellectual agenda of science. We expect our authors to comply with, best practice in publication ethics as well as in quality of their articles.

Withdrawal of a manuscript will be permitted only for the most compelling and unavoidable reasons. For withdrawal of a manuscript authors need to submit an "Article withdrawal Form", signed by all authors mentioning the reason for withdrawal to the Editorial Office. The form is available from the web page of the journal. Authors must not assume that their manuscript has been withdrawn until they have received appropriate notification to this effect from the editorial office.

In a case where a manuscript has taken more than five months' time for review process, that allows the author to withdraw manuscript.

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If the reason finds to be acceptable, the author is allowed to withdraw the manuscript without paying any withdrawal penalty. If not the Journal of Inonu Liver Transplantation Institute will not accept any manuscripts from the same author for one year.

Important notes: Manuscripts may be withdrawn at any stage of review and publication process by submitting a request to the editorial office. Manuscript withdrawal will be permitted after submission only for the most compelling and unavoidable reasons.

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The manuscript will not be withdrawn from publication process until a completed, signed form is received by the editorial office. Authors must not assume that their manuscript has been withdrawn until they have received appropriate notification to this effect from the Journal of Inonu Liver Transplantation Institute editorial office.

Retraction Policy

The publisher will take all appropriate measures to modify the article in question, in close cooperation with the editors, in cases of alleged or proven scientific misconduct, fraudulent publication, or plagiarism. This includes the prompt publication of an erratum, disclosure, or retraction of the affected work in the most severe case. Together with the editors, the publisher will take reasonable steps to detect and prevent the publication of articles in which research misconduct occurs and will under no circumstances promote or knowingly allow such abuse to occur.

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Appeal and complaint cases are handled within the scope of COPE guidelines by the Editorial Board of the journal. Appeals should be based on the scientific content of the manuscript. The final decision on the appeal and complaint is made by Editor in Chief. An Ombudsperson or the Ethical Editor is assigned to resolve cases that cannot be resolved internally. Authors should get in contact with the Editor in Chief regarding their appeals and complaints via e-mail at kare@karepb.com.

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.

Reviews are typically completed within one month of submission to the journal. Authors will be sent constructive reviewer comments intended to be useful. In general, the instructions, objections, and requests made by the reviewers should be followed. The revised manuscript should clearly and precisely indicate every step taken in accordance with the reviewers' notes. A list of responses and the corrections made to each comment should be provided.

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.

ORCID ID

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/

PLAGIARISM DETECTION

All submissions are screened using similarity detection software at least two times: on submission and after completing revisions. In the event of alleged or suspected research misconduct, e.g., plagiarism, citation manipulation, or data falsification/fabrication, the editorial board will follow and act in accordance with COPE guidelines. Plagiarism, including self-plagiarism, that is detected at any stage will result in rejection of the manuscript.

PUBLICATION FEE - CHARGES

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, case report guidelines (CARE) 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: A "Letter to the Editor" is a type of manuscript that discusses important or overlooked aspects of a previously published article. This type of manuscript may also present articles on topics within the scope of the journal that are of interest to readers, particularly educational cases. Additionally, readers may use the "Letter to the Editor" format to share comments on published manuscripts.

Key Features:

- The "Letter to the Editor" should be unstructured and should not include an abstract, keywords, tables, figures, images, or other media.
- The manuscript being commented on must be properly cited within the "Letter to the Editor."
- Our journal considers all feedback on published articles. However, we emphasize that comments should be scientifically relevant and meaningful to the discussion. Irrelevant or unfounded comments may be rejected.

ICMJE Guidelines:

Our journal adheres to the guidelines set forth by the ICMJE (International Committee of Medical Journal Editors). According to ICMJE, "Letters to the Editor" should be a platform for responsible debate, critique, and discussion. These letters may raise substantial criticisms or questions about previously published articles, and authors of the discussed articles are expected to respond to these criticisms.

ICMJE also notes that editors have the right to edit these letters for length, grammar, and style. However, all letters should contribute constructively to the academic discussion and critique, and those deemed irrelevant or unfounded may be rejected.

You can view the ICMJE guidelines on "Correspondence" here.

Table 1. Limitations for each manuscript type.

Type of manuscript	Wordlimit	Abstract word limit	Reference limit	Table limit	Figure limit
Original Article	4000-5000	350-400	40-50	6	6
Review Article	5000-6000	350-400	50-60	6	10
Meta analysis	5000	350	50	6	10
Case 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 350*400 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.
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Review

EMT and Inflammation: The Case of Portal Vein Thrombosis

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Abstract

Hepatocellular Carcinoma (HCC) is the primary malignancy of the liver, which is typically associated with underlying chronic liver diseases, such as cirrhosis, hepatitis B, or hepatitis C infections. Inflammation is a prevailing concern for liver disorders with documented impacts on the tumorigenic and metastatic propensities of individuals afflicted with HCC. Portal vein tumor thrombus (PVTT) is a widespread problem among patients with HCC, manifesting in approximately 50% of cases. A comprehensive understanding of the PVTT mechanism is imperative to comprehend and address the challenges associated with HCC progression. The intricate nature of the mechanism underlying PVTT formation and its influence on metastasis progression remains to be fully elucidated. Given that the portal venous system's microenvironment is conducive to tumor cells' survival and further metastasis, a critical exploration of the associations and parallels among metastasis, epithelial-mesenchymal transition (EMT), and PVTT becomes paramount. Signaling pathways play diverse roles in the progression of various diseases, with particular significance attributed to their role in HCC advancement, which highlights the necessity for precise determination of their effects in the context of HCC and PVTT. We highlight the significance of understanding the interplay between EMT, inflammation, and PVTT in the HCC context. Furthermore, we revisit the signaling pathways impacting this interconnected network, providing useful perspectives to support research initiatives. This review aims to guide research studies toward promising outcomes in exploring HCC complexities by defining the possible association between PVTT and EMT, thus identifying potential target areas for advanced therapeutics.

Keywords: Hepatocellular carcinoma, portal vein tumor thrombus, Inflammation, Epithelial to mesenchymal transition, signaling pathway.

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Hepatocellular carcinoma (HCC) stands as the predominant liver cancer globally, representing approximately 90% of liver cancer cases. The prognosis of HCC is closely tied to the tumor stage, with early diagnosis over 70% survival rates at five years, contrasting starkly with less than 20% for advanced stages within the same timeframe.[1] Life expectancy for HCC patients hinges on the cancer diagnosis and stage, prevalent survival is poor, 5-year relative survival rate is 18.4%. The 5-year survival rates reach 33% in patients with localized, 10% in patients with regional, and 2% in with metastatic disease.[2] At an early stage of HCC, surgical resection, liver transplant, and local ablation can increase the survival of the patients, whereas at an advanced stage, chemotherapy has no satisfactory effect, and this situation leads to a poor prognosis.[3] One of the most popular treatment methods for patients with intermediate-stage, well-preserved liver function, and unresectable multinodular lesions, who lack vascular invasion or extra-

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hepatic spread is Transarterial chemoembolization (TACE). However, TACE is not recommended for advanced-stage HCC patients. Immune checkpoint inhibition (ICI) has been successful in treating cancer patients, including those with HCC, by improving efficacy and safety and enhancing long-term survival outcomes. A combination of two ICIs targeting different immune checkpoint pathways is considered in clinical trials.[4] The etiology of HCC is primarily linked to environmental, dietary, or lifestyle factors, with the interplay between these factors and genetic elements giving rise to its development. Significant risk factors include liver cirrhosis, hepatitis B virus (HBV) and hepatitis C virus (HCV) infection, excessive alcohol consumption, aflatoxin B1 ingestion, nonalcoholic steatohepatitis (NASH), perinatal transmission and maternal viral load.[5] HCC exhibits associations with various parameters and occasionally arises on a previously compromised liver, often in the context of chronic hepatopathy or cirrhosis. Additionally, hereditary diseases such as hemochromatosis, Wilson's disease, and a-1-antitrypsin deficiency may influence the HCC process.[6]

Inflammation and HCC

Infection typically elicits inflammation as a well-known response; however, this immune reaction does not always confer complete protection. In certain instances, such as in the context of cancer, infections can result in non-resolving inflammation due to the host's defense mechanisms. The progression of various cancer types is profoundly influenced by the tumor microenvironment, orchestrated through signaling pathways crucial for proliferation and survival.[6,7] Within the domain of liver diseases, inflammation is a significant concern, notably amplifying the tumorigenic and metastatic capabilities of HCC patients. Since HCC is the predominant type of liver cancer and is significantly impacted by inflammation, it is categorized as an inflammation-linked cancer, primarily manifesting in situations of inflammation or hepatic injury.[8]

Numerous studies revealed that deregulated microenvironments substantially impact tumorigenesis.[9] Liver inflammation ensues upon exposure to various substances, inducing microenvironmental changes and triggering inflammatory cascades. Factors such as HBV, HCV, diabetes, obesity, excessive alcohol consumption, and metabolic diseases are principal contributors to inflammation. These factors predominantly lead to fibrosis and cirrhosis, consequently propelling the progression of HCC (Fig. 1).[8]

EMT and Metastasis in HCC Progression

Epithelial–mesenchymal transition (EMT) is a cellular process during which epithelial cells acquire mesenchymal characteristics, achieved by the upregulation of mesenchymal related markers and the downregulation of epi-

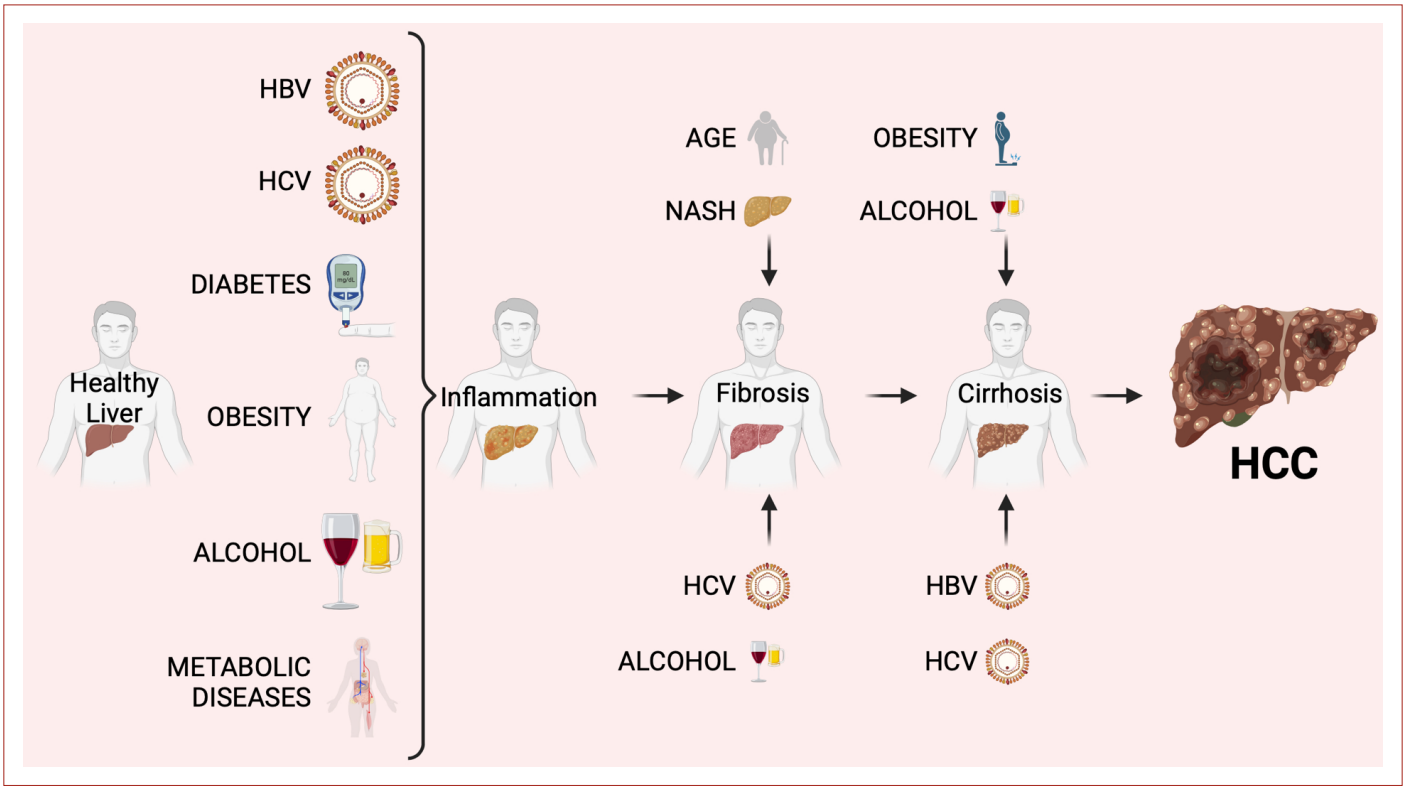

Figure 1. Microenvironmental changes lead to inflammation, fibrosis, and cirrhosis, consequently triggering the progression of HCC.

thelial markers. A bona-fide EMT is characterized by the well-known cadherin switch, where the down regulation of E-cadherin is compensated by the upregulation of N-cadherin.[10] This is associated with apparent changes in cellular phenotypes of epithelial cells, such as loss of apical–basal polarity, modulation of the cytoskeleton, and decrease in cell–cell adhesive properties. During the EMT process, cells may either individually or collectively gain mesenchymal characteristics and increase the ability of invasion and motility. The accumulated loss or gain of epithelial/mesenchymal (E/M) characteristics drives a cell toward diverse intermediate states in a fluid and reversible manner between a complete epithelial and a complete mesenchymal state usually referred to as epithelial mesenchymal plasticity (EMP).[11]

Throughout the evolution of multicellular organisms, various phenomena have shaped their development, including the emergence of specialized cell types and the diversification of cellular phenotypes. EMT is central to this divergence of cell phenotypes, a critical process observed in early development and normal cell differentiation. EMT is the primary mechanism facilitating the organization of highly specialized tissues and organ systems in diverse organisms. Given its significant roles in organismal development, several key components of molecular pathways associated with the EMT process have been identified, including Zeb1/2, Snail/Slug, Twist, Six1, Cripto, and TGFβ.[12] EMT is vital in various physiological and pathological processes such as embryogenesis, inflammation, fibrosis, wound healing, and cancer development, and is classified into three EMT-types accordingly. Recent studies have underscored the impact of EMT on the progression of metastasis in carcinogenic tumors.[13,14] EMT, a highly conserved program in developmental contexts, assumes a dual role in cancer. While its reactivation enables cancer cells to acquire malignant traits such as mobility, resistance to apoptotic stimuli, invasiveness, increased tumor stemness, and heightened resistance to chemotherapy and immunotherapy, it fundamentally contributes to the metastasis process.[15]

In the context of cancer progression, the description of hybrid E/M states have gained wide attention. This fluid state is largely observed in circulating tumor cells (CTCs) associated with various types of cancers and their metastases. This is clearly a manifestation of the complexity of the interplay between tumor biology, its microenvironment, and EMP. Which can be considered as a major cause of tumor heterogeneity.[16,17] The phenotypic heterogeneity of these various hybrid E/M states depends on the tissue of origin of the tumor cell, specific combinations of expressed EMT-TFs, and chromatin modifications.[11]

The development and progression of HCC involve intricate processes encompassing chronic inflammation, tissue remodeling, genetic changes, and modifications in cellular signaling. Within this complex scenario, microenvironmental factors play a pivotal role in modulating these crucial processes, with inflammatory components emerging as the foremost contributors to tumor proliferation, angiogenesis, and metastasis.[8] Metastasis poses the most formidable challenge in cancer treatment, constituting a complex process involving diverse pathways and cascades. The intricacy of metastasis extends beyond the intrinsic characteristics of tumor cells, encompassing the microenvironment as a critical driver of key factors. The involvement of infiltrated immune cells is crucial in the metastatic process, with their secreted cytokines, chemokines, and growth factors playing decisive roles in conferring metastatic and invasive properties to cancer cells.[18,19] HCC tends to invade vascular structures. Macrovascular invasion refers to the macroscopically observable tumor in the vein, however microvascular invasion (MVI) refers to the presence of tumor cells inside portal or hepatic venous systems.[20] In addition to the macrometastasis process, patients with advanced stages of HCC have high tumor recurrence rates after resection or orthotopic liver transplantation. This can be considered as a consequence of the tumor in other parts of the liver, positive resection margins, tumor invasion of the hepatic vasculature, and microscopic metastasis, collectively leading to the consideration of HCC as a micrometastatic disease.[21]

In the metastatic process, the EMT program can induce cancer cells to generate proinflammatory factors. Tumorigenesis involves the production of various matrix metalloproteinases (MMPs), cytokines, and chemokines. This production cascade is initiated to provoke a response against danger signals emanating from the tumor. The production process involves different cell types, including cancer, dendritic, cancer-associated fibroblasts, and endothelial cells. In the progression of HCC, the transcription factors Snail, Slug, Twist, and Zeb are upregulated, significantly promoting EMT. Previous research has highlighted the involvement of EMT-related exosomes, microRNAs, long non-coding RNAs, and regulatory signaling pathways in HCC progression. The associations between EMT-related mechanisms and HCC progression underline the role of EMT and point to a promising new avenue for targeted therapy.[22–24]

Portal Vein Tumor Thrombus (PVTT): Understanding its Impact on HCC Progression

In HCC patients, PVTT poses a significant challenge for HCC treatment, occurring in approximately 50% of cases.[25] This complication is significantly linked to poor liver function,

aggressive tumor biology, elevated tumor number and size, and heightened levels of serum markers such as AFP.[26] The portal venous system plays a pivotal role in HCC progression; when the primary tumor infiltrates this system, HCC can extend into portal vein branches, leading to inconspicuous intrahepatic metastasis within neighboring liver sections. This mechanism notably impacts the early intrahepatic recurrence. The prognosis of HCC patients exhibits considerable variation and experiences a drastic decline with the presence of PVTT. Without PVTT, a 5-year survival rate of around 18%, while in the presence of PVTT, it diminishes to a mere 2.7 to 4 months.[27,28]

Consequently, PVTT demonstrates diverse associations with cancer progression, potentially playing an essential role in inflammation and metastasis. A specific study focused on inflammatory biomarkers and the presence of PVTT revealed significant increases in inflammation markers, including the neutrophil-to-lymphocyte ratio (NLR), platelet count-to-lymphocyte ratio (PLR), erythrocyte sedimentation rate (ESR), and gamma-glutamyl transpeptidase (GGTP) in patients with PVTT compared to those without.[29] Another study delving into inflammatory cells and metastasis highlighted the facilitative role of inflammatory cells and their mediators in tumor cell invasion, extravasation, and metastatic outgrowth.[30] A noteworthy finding in patients with cirrhosis revealed that markers of systemic inflammation, specifically IL-6 and lymphopenia, predict PVTT independently of markers of portal hypertension, shedding light on a previously overlooked factor (IL-6) in the pathogenesis of portal vein thrombosis (PVT).[31] Furthermore, research has emphasized the pivotal role of systemic inflammation in portal vein thrombosis, with interleukin 6, a significant inflammatory cytokine, independently associated with this complication. The correlation between interleukin-6 levels and PVT necessitates further examination.[32]

Major Signaling Pathways in EMT and PVTT: Unraveling Cellular Associations

Signaling pathways play a central role in preserving diverse cellular contexts. Beyond their fundamental functions, they serve as conduits that elucidate intricate cellular associations, enabling the definition of interactions and their consequences. Key signaling pathways implicated in EMT include TGFβ, Wnt, Notch, and Hedgehog.[33]

TGFβ Signaling Pathway: Bridging EMT and PVTT

TGFβ is a cytokine secreted with significant roles, including regulating cell proliferation, migration, and differentiation across various cell types. In cancer progression, the late stage undergoes substantial changes that impact functional pathways. Cancer cells maintain responsiveness to TGFβ but develop resistance to its cytostatic effects, making TGFβ a key promoter of tumorigenesis by inducing EMT.[34] TGFβ is a potent EMT inducer linked to crucial signaling pathways, such as the Wnt/β-catenin signaling pathway. It has been extensively studied in the context of metastasis and EMT, and its role in cancer-related inflammation needs precise definition.[35,36] The TGFβ pathway impacts metastasis and EMT in the cancer scenario and is essential to inflammation, particularly for HCC. Studies indicate that HBV infection and the activity of the TGFβ-miR-34a-CCL22 axis act as potent etiological factors predisposing HCC patients to develop PVTT.[37] Another research study delineated the effect of the TGFβ pathway on PVTT, associating the imbalance of TGFβ1/BMP-7 pathways with large tumor size, microvascular invasion, PVTT, and poor differentiation.[38]

Wnt/β-catenin Signaling Pathway: Orchestrating EMT and PVTT

The Wnt/β-catenin signaling pathway is crucial in various cellular functions, including proliferation, development, differentiation, and disease progression. Research studies illustrate the involvement of the Wnt/β-catenin signaling pathway in the EMT process. The Wnt/β-catenin pathway has a dual role in mediating EMT. It was shown to induce EMT via miR-300.[39] In contrast, inhibition of Wnt signaling through siRNAs targeting the long non-coding RNA UCA1 was shown to inhibit EMT.[40]

Additionally, studies have indicated that microRNA-136 serves as an activator of both Wnt signaling and EMT, establishing a clear association between the Wnt/β-catenin signaling pathway and EMT.[41] In various human malignancies, the upregulation of different microRNAs (miRNAs) has been observed, and these miRNAs are closely linked to critical functions such as cell proliferation, differentiation, migration, invasion, and apoptosis.[42] Generally, PVTT is considered a distinct type of metastasis in HCC. In this context, altered miRNAs may contribute to PVTT development by mediating the genes involved in tumor metastasis. Recent studies on PVTT have established a connection between the Wnt/β-catenin pathway and PVTT formation through specific microRNAs. The upregulation of miR-517b-3p recently activated the Wnt/β-catenin signaling pathway, increasing cell proliferation, migration, and invasion, thus promoting PVTT.[43,44]

Notch Signaling Pathway: Balancing Proliferation and Apoptosis in HCC

The Notch signaling pathway, a conserved mechanism maintaining a delicate equilibrium between cell proliferation and apoptosis, plays a crucial role in developing and progressing various malignancies.[45] Its significance ex-

tends to HCC progression, influencing neoplastic growth, invasion capability, and stem-like properties. Despite this, a comprehensive understanding of the deregulated expression of individual Notch receptors and ligands and resulting phenotypic changes still need to be improved in the HCC.[46] The Notch coactivator MAML1 has been identified as a contributor to the aggressiveness of different cancer types, including HCC.[47] Recent studies have highlighted the association between aberrant Notch1 expression and HCC metastatic disease through the Notch1-Snail1-E-cadherin pathway. Notably, the knockdown of Notch1 reversed HCC tumor metastasis in a mouse model.[48]

Furthermore, investigations revealed that the CD90 biomarker was exclusively detected in HCC patient samples, indicating a correlation of this biomarker with poor prognosis. Isolated CD90+ populations from HCC cell lines exhibited increased tumorigenicity, chemoresistance, tumor invasion, and metastasis through Notch signaling activation.[49] The literature on HCC lacks a comprehensive explanation for the association between Notch signaling and PVTT, yet assuming an association between Notch signaling and EMT leading to PVTT is plausible. An in-depth understanding of the critical role of EMT or similarities in PVTT formation and a better comprehension of mechanistic regulation opens new avenues for developing potential therapeutic targets of clinical importance.

Hedgehog Signaling Pathway: Navigating EMT and PVTT Complexity

The hedgehog (Hh) signaling pathway, a foundational and conserved route, is pivotal in governing embryonic development and tissue repair. Its activation during development regulates the EMT process. Aberrant activation of the Hh signaling pathway assumes various roles, including malignant transformation, progression, drug resistance, and metastatic processes in several solid tumors, including HCC.[50] Overexpression of Hh protein markers in liver tumor tissues correlates with aggressive features and poor survival. Multiple studies have demonstrated that the Hh pathway sustains tumor growth, metastasis, and a mesenchymal phenotype.[51,52] In HCC patients, the predominant cause of death is local invasion and metastasis in other organs. Research indicates that mRNA levels of the receptor (PTCH1) and GLI1, both Hh components, indicate recurrence after resection.[50] Given that PVTT is considered an HCC-specific metastasis, the Hh signaling pathway may exhibit a complex association with the PVTT mechanism, akin to EMT.

While various signal transduction or molecular pathways associated with PVTT have been identified, the precise mechanisms of PVTT remain largely unknown [78]. Analyses of dysregulated genes between HCC and PVTT suggest that extracellular matrix receptor interaction correlates with HCC Field venous metastases.[25,26] Other factors proposed to contribute to PVTT development include vascular endothelial cells, immune cells in the tumor microenvironment, genomic irregularities, sequential alterations of mRNA expression, DNA methylation of differentially expressed genes, cancer stem cells, dysregulation of extracellular matrix organization, and focal adhesion.[45,53] The complete mechanisms of PVTT formation have mostly remained unidentified. Traditionally, it was believed that PVTT forms following the direct invasion of a liver tumor, resulting in a hepatic artery-portal vein fistula and portal vein countercurrent. However, recent studies have detected a distinct PVTT (dPVTT), a noteworthy type of PVTT distant from the liver tumor nodule. Comparative proteomics studies revealed that dPVTT-owned molecular signatures are different from those of liver tumors, suggesting that the mechanism of PVTT formation is more complex than previously thought.[54,55]

Conclusion

In conclusion, HCC poses a significant global health burden, representing the majority of liver cancer cases with varying prognoses dependent on tumor stage. Early detection significantly improves survival rates, emphasizing the critical role of timely intervention. The multifaceted etiology of HCC involves intricate interplays between environmental, dietary, lifestyle factors, and genetic elements. Inflammation is pivotal in HCC progression, amplifying tumorigenic and metastatic capabilities. The complex processes of metastasis, particularly the EMT, underscore the complexity of HCC development.

Of notable concern is the common occurrence of PVTT in HCC patients, linked to poor liver function and aggressive tumor biology. PVTT profoundly impacts patient prognosis, with a substantial decline in survival rates. Inflammatory markers, such as the neutrophil-to-lymphocyte ratio and interleukin-6, exhibit significant associations with PVTT, shedding light on their potential role as predictive indicators. The involvement of major signaling pathways, including TGFβ, Wnt/β-catenin, Notch, and Hedgehog, further complicates the intricate network governing EMT and PVTT.

While substantial progress has been made in understanding the roles of these signaling pathways in HCC progression, their precise implications in PVTT formation still need to be discovered. The complexity of PVTT mechanisms, involving factors such as extracellular matrix interactions, immune cells, genomic irregularities, and cancer stem cells,

poses a challenge for comprehensive elucidation. Recent studies challenging traditional beliefs about PVTT formation highlight the need for further exploration into the distinct molecular signatures of PVTT, paving the way for more targeted therapeutic approaches.

Unraveling the enigma surrounding PVTT is crucial in advancing our understanding of liver cancer progression. Continued research into the specific contributions of inflammatory markers and signaling pathways to PVTT formation promises to reveal new therapeutic avenues, ultimately improving the diagnosis and treatment of HCC.

Disclosures

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References

1. Calderon-Martinez E, Landazuri-Navas S, Vilchez E, Cantu-Hernandez R, Mosquera-Moscoso J, Encalada S, et al. Prognostic Scores and Survival Rates by Etiology of Hepatocellular Carcinoma: A Review. J Clin Med Res [Internet]. 2023 [cited 2023 Nov 6];15(4):200. Available from: /pmc/articles/PMC10181349/
2. Puisieux MF, Pellat A, Assaf A, Ginestet C, Brezault C, Dhooge M, et al. Therapeutic Management of Advanced Hepatocellular Carcinoma: An Updated Review. Cancers (Basel) [Internet]. 2022 May 1 [cited 2023 Dec 8];14(10). Available from: /pmc/articles/PMC9139863/
3. Liu JKH, Irvine AF, Jones RL, Samson A. Immunotherapies for hepatocellular carcinoma. Cancer Med [Internet]. 2022 Feb 1 [cited 2023 Jun 20];11(3):571–91. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/cam4.4468
4. van Bömmel F, Berg T, Lordick F. Immune checkpoint inhibition (ICI) in current systemic therapies for hepatocellular carcinoma (HCC). ESMO Gastrointestinal Oncology [Internet]. 2023 Oct 1 [cited 2023 Dec 8];1:27–39. Available from: http://www.esmogastro.org/article/S2949819823000055/fulltext
5. António Gomes M, Gonçalves Priolli D, Guilherme Tralhão J, Filomena Botelho M. Hepatocellular carcinoma: epidemiology, biology, diagnosis, and therapies. Revista da Associação Médica Brasileira (English Edition) [Internet]. 2013 Jan 1 [cited 2023 Jun 20];59(5):514–24. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2255482313705113
6. Anzola M. Hepatocellular carcinoma: role of hepatitis B and hepatitis C viruses proteins in hepatocarcinogenesis. J Viral Hepat [Internet]. 2004 Sep [cited 2023 Jun 20];11(5):383–93. Available from: https://pubmed.ncbi.nlm.nih.gov/15357643/
7. Sengez B, Carr BI, Alotaibi H. EMT and Inflammation: Crossroads in HCC. J Gastrointest Cancer [Internet]. 2023 Mar 1 [cited 2023 Aug 4];54(1):204–12. Available from: https://pubmed.ncbi.nlm.nih.gov/35020133/
8. Grazia Refolo M, Messa C, Guerra V, Irving Carr B. cancers Inflammatory Mechanisms of HCC Development. 2020 [cited 2023 Jun 20]; Available from: www.mdpi.com/journal/cancers
9. Multhoff G, Molls M, Radons J. Chronic inflammation in cancer development. Front Immunol [Internet]. 2012 [cited 2023 Jun 20];2(JAN). Available from: https://pubmed.ncbi.nlm.nih.gov/22566887/
10. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest [Internet]. 2009 Jun 1 [cited 2023 Jun 22];119(6):1420–8. Available from: https://pubmed.ncbi.nlm.nih.gov/19487818/
11. Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, et al. Guidelines and definitions for research on epithelial–mesenchymal transition. Nature Reviews Molecular Cell Biology 2020 21:6 [Internet]. 2020 Apr 16 [cited 2025 Apr 21];21(6):341–52. Available from: https://www.nature.com/articles/s41580-020-0237-9
12. Chen T, You Y, Jiang H, Wang ZZ. Epithelial-mesenchymal transition (EMT): A biological process in the development, stem cell differentiation, and tumorigenesis. J Cell Physiol [Internet]. 2017 Dec 1 [cited 2023 Jun 22];232(12):3261–72. Available from: https://pubmed.ncbi.nlm.nih.gov/28079253/
13. Thiery JP. Epithelial-mesenchymal transitions in development and pathologies. Curr Opin Cell Biol [Internet]. 2003 [cited 2023 Jun 20];15(6):740–6. Available from: https://pubmed.ncbi.nlm.nih.gov/14644200/
14. Micalizzi DS, Farabaugh SM, Ford HL. Epithelial-Mesenchymal Transition in Cancer: Parallels Between Normal Development and Tumor Progression. J Mammary Gland Biol Neoplasia [Internet]. 2010 Jun [cited 2023 Jun 20];15(2):117. Available from: /pmc/articles/PMC2886089/
15. Mittal V. Epithelial Mesenchymal Transition in Tumor Metastasis. https://doi.org/101146/annurev-pathol-020117-043854 [Internet]. 2018 Jan 24 [cited 2023 Jun 22];13:395–412. Available from: https://www.annualreviews.org/doi/abs/10.1146/annurev-pathol-020117-043854
16. Schliekelman MJ, Taguchi A, Zhu J, Dai X, Rodriguez J, Celiktas M, et al. Molecular portraits of epithelial, mesenchymal, and hybrid States in lung adenocarcinoma and their relevance to survival. Cancer Res [Internet]. 2015 May 1 [cited 2025 Apr 21];75(9):1789–800. Available from: https://pubmed.ncbi.nlm.nih.gov/25744723/
17. Jolly MK, Boareto M, Huang B, Jia D, Lu M, Onuchic JN, et al. Implications of the Hybrid Epithelial/Mesenchymal Phenotype in Metastasis. Front Oncol [Internet]. 2015 [cited 2025 Apr 21];5(JUN). Available from: https://pubmed.ncbi.nlm.nih.gov/26258068/
18. Wu Y, Zhou BP. Inflammation: a driving force speeds cancer metastasis. Cell Cycle [Internet]. 2009 Oct 10 [cited 2023 Jun 20];8(20):3267. Available from: /pmc/articles/PMC3702728/

19. Zhao H, Wu L, Yan G, Chen Y, Zhou M, Wu Y, et al. Inflammation and tumor progression: signaling pathways and targeted intervention. [cited 2023 Jun 20]; Available from: https://doi.org/10.1038/s41392-021-00658-5
20. Tang A. Using MRI to assess microvascular invasion in hepatocellular carcinoma. Radiology [Internet]. 2020 Dec 1 [cited 2023 Dec 8];297(3):582–3. Available from: https://pubs.rsna.org/doi/10.1148/radiol.2020203376
21. Kar S, Carr BI. Detection of liver cells in peripheral blood of patients with advanced-stage hepatocellular carcinoma. Hepatology [Internet]. 1995 Feb 1 [cited 2023 Dec 8];21(2):403–7. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/hep.1840210222
22. Suarez-Carmona M, Lesage J, Cataldo D, Gilles C. EMT and inflammation: inseparable actors of cancer progression. Mol Oncol [Internet]. 2017 Jul 1 [cited 2023 Jul 4];11(7):805–23. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/1878-0261.12095
23. Crusz SM, Balkwill FR. Inflammation and cancer: advances and new agents. Nat Rev Clin Oncol [Internet]. 2015 Oct 24 [cited 2023 Jul 4];12(10):584–96. Available from: https://pubmed.ncbi.nlm.nih.gov/26122183/
24. Puisieux A, Brabletz T, Caramel J. Oncogenic roles of EMT-inducing transcription factors. Nature Cell Biology 2014 16:6 [Internet]. 2014 May 30 [cited 2023 Jul 5];16(6):488–94. Available from: https://www.nature.com/articles/ncb2976
25. Zhang ZM, Lai ECH, Zhang C, Yu HW, Liu Z, Wan BJ, et al. The strategies for treating primary hepatocellular carcinoma with portal vein tumor thrombus. International Journal of Surgery. 2015 Aug 1;20:8–16.
26. Khan AR, Wei X, Xu X. Portal Vein Tumor Thrombosis and Hepatocellular Carcinoma – The Changing Tides. J Hepatocell Carcinoma [Internet]. 2021 Sep [cited 2023 Nov 6];8:1089. Available from: /pmc/articles/PMC8434852/
27. Ye JZ, Wang YY, Bai T, Chen J, Xiang B De, Wu FX, et al. Surgical resection for hepatocellular carcinoma with portal vein tumor thrombus in the Asia-Pacific region beyond the Barcelona Clinic Liver Cancer treatment algorithms: a review and update. Oncotarget [Internet]. 2017 Nov 11 [cited 2023 Aug 3];8(54):93258. Available from: /pmc/articles/PMC5696262/
28. Sun H, Yang H, Mao Y. Personalized treatment for hepatocellular carcinoma in the era of targeted medicine and bioengineering. Front Pharmacol. 2023 May 5;14:1150151.
29. Carr BI, Guerra V, Donghia R. Portal Vein Thrombosis and Markers of Inflammation in Hepatocellular Carcinoma. J Gastrointest Cancer [Internet]. 2020 Dec 1 [cited 2023 Aug 4];51(4):1141–7. Available from: https://pubmed.ncbi.nlm.nih.gov/32851544/
30. Qian BZ, Pollard JW. Macrophage Diversity Enhances Tumor Progression and Metastasis. Cell [Internet]. 2010 Apr 4 [cited 2023 Aug 4];141(1):39. Available from: /pmc/articles/PMC4994190/
31. Nery F, Carneiro P, Correia S, Macedo C, Gandara J, Lopes V, et al. Systemic inflammation as a risk factor for portal vein thrombosis in cirrhosis: A prospective longitudinal study. Eur J Gastroenterol Hepatol [Internet]. 2021 Dec 1 [cited 2023 Nov 6];33(1):E108–13. Available from: https://journals.lww.com/eurojgh/fulltext/2021/12001/systemic_inflammation_as_a_risk_factor_for_portal.12.aspx
32. Huang X, Fan X, Zhang R, Jiang S, Yang K, Chen S. Systemic inflammation and portal vein thrombosis in cirrhotic patients with gastroesophageal varices. Eur J Gastroenterol Hepatol [Internet]. 2020 Mar 1 [cited 2023 Nov 6];32(3):401–5. Available from: https://journals.lww.com/eurojgh/fulltext/2020/03000/systemic_inflammation_and_portal_vein_thrombosis.17.aspx
33. Liu X, Yun F, Shi L, Li ZH, Luo NR, Jia YF. Roles of Signaling Pathways in the Epithelial-Mesenchymal Transition in Cancer. Asian Pac J Cancer Prev [Internet]. 2015 [cited 2023 Aug 9];16(15):6201–6. Available from: https://pubmed.ncbi.nlm.nih.gov/26434817/
34. Hao Y, Baker D, Dijke P Ten. TGF-β-Mediated Epithelial-Mesenchymal Transition and Cancer Metastasis. Int J Mol Sci [Internet]. 2019 Jun 1 [cited 2023 Aug 15];20(11). Available from: /pmc/articles/PMC6600375/
35. Fuxe J, Karlsson MCI. TGF-β-induced epithelial-mesenchymal transition: a link between cancer and inflammation. Semin Cancer Biol [Internet]. 2012 Oct [cited 2023 Aug 15];22(5–6):455–61. Available from: https://pubmed.ncbi.nlm.nih.gov/22627188/
36. Zhou C, Liu J, Tang Y, Liang X. Inflammation linking EMT and cancer stem cells. Oral Oncol. 2012 Nov 1;48(11):1068–75.
37. Yang P, Li QJ, Feng Y, Zhang Y, Markowitz GJ, Ning S, et al. TGF-β-miR-34a-CCL22 Signaling-Induced Treg Cell Recruitment Promotes Venous Metastases of HBV-Positive Hepatocellular Carcinoma. Cancer Cell [Internet]. 2012 Sep 9 [cited 2023 Aug 15];22(3):291. Available from: /pmc/articles/PMC3443566/
38. Ning J, Ye Y, Bu D, Zhao G, Song T, Liu P, et al. Imbalance of TGF-β1/BMP-7 pathways induced by M2-polarized macrophages promotes hepatocellular carcinoma aggressiveness. Molecular Therapy. 2021 Jun 2;29(6):2067–87.
39. Zhang JQ, Chen S, Gu JN, Zhu Y, Zhan Q, Cheng DF, et al. MicroRNA-300 promotes apoptosis and inhibits proliferation, migration, invasion and epithelial-mesenchymal transition via the Wnt/β-catenin signaling pathway by targeting CUL4B in pancreatic cancer cells. J Cell Biochem [Internet]. 2018 Jan 1 [cited 2025 Apr 21];119(1):1027–40. Available from: https://pubmed.ncbi.nlm.nih.gov/28685847/
40. Xiao C, C.-H. W, H.-Z. H. LncRNA UCA 1 promotes epithelial-mesenchymal transition (EMT) of breast cancer cells via enhancing Wnt / beta-catenin signaling pathway. 2016;
41. Tanabe S. Wnt Signaling and Epithelial-Mesenchymal Transition Network in Cancer. Res J Oncol [Internet]. 2018 [cited 2023 Aug 9];2(2):3. Available from: http://www.imedpub.com/
42. Jiang ZL, Zhang FX, Zhan HL, Yang HJ, Zhang SY, Liu ZH, et al. miR-181b-5p Promotes the Progression of Cholangiocarcinoma by Targeting PARK2 via PTEN/PI3K/AKT Signaling Pathway. Biochem

Genet [Internet]. 2022 Feb 1 [cited 2025 Apr 21];60(1):223–40. Available from: https://pubmed.ncbi.nlm.nih.gov/34169384/

43. Elhendawy M, Abdul-Baki EA, Abd-Elsalam S, Hagras MM, Zidan AA, Abdel-Naby AY, et al. MicroRNA signature in hepatocellular carcinoma patients: identification of potential markers. Mol Biol Rep [Internet]. 2020 Jul 1 [cited 2023 Aug 9];47(7):4945–53. Available from: https://pubmed.ncbi.nlm.nih.gov/32430845/
44. Ke RS, Huang KZ, Bao D sheng, Yang JR, Lv LZ, Jiang Y, et al. miR-517b-3p promotes the progression of portal vein tumor thrombus via activating Wnt/β-catenin signaling pathway in Hepatocellular carcinoma. 2022 [cited 2023 Aug 9]; Available from: https://doi.org/10.21203/rs.3.rs-1468856/v1
45. Wang D, Zhu Y, Tang J, Lian Q, Luo G, Wen W, et al. Integrative molecular analysis of metastatic hepatocellular carcinoma. BMC Med Genomics [Internet]. 2019 Nov 13 [cited 2023 Nov 6];12(1). Available from: https://pubmed.ncbi.nlm.nih.gov/31722693/
46. Giovannini C, Fornari F, Piscaglia F, Gramantieri L. Notch Signaling Regulation in HCC: From Hepatitis Virus to Non-Coding RNAs. Cells [Internet]. 2021 Mar 1 [cited 2023 Aug 15];10(3):1–21. Available from: /pmc/articles/PMC8000248/
47. Shen H, McElhinny AS, Cao Y, Gao P, Liu J, Bronson R, et al. The Notch coactivator, MAML1, functions as a novel coactivator for MEF2C-mediated transcription and is required for normal myogenesis. Genes Dev [Internet]. 2006 Mar 15 [cited 2023 Aug 15];20(6):675–88. Available from: http://genesdev.cshlp.org/content/20/6/675.full
48. Wang XQ, Zhang W, Lui ELH, Zhu Y, Lu P, Yu X, et al. Notch1-Snail1-E-cadherin pathway in metastatic hepatocellular carcinoma. Int J Cancer [Internet]. 2012 Aug 1 [cited 2023 Aug 15];131(3):E163–72. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/ijc.27336
49. Liu YC, Yeh CT, Lin KH. Cancer Stem Cell Functions in Hepatocellular Carcinoma and Comprehensive Therapeutic Strategies. Cells 2020, Vol 9, Page 1331 [Internet]. 2020 May 26 [cited 2023 Aug 15];9(6):1331. Available from: https://www.mdpi.com/2073-4409/9/6/1331/htm
50. Ding J, Li HY, Zhang L, Zhou Y, Wu J. Hedgehog Signaling, a Critical Pathway Governing the Development and Progression of Hepatocellular Carcinoma. Cells [Internet]. 2021 Jan 1 [cited 2023 Aug 10];10(1):1–18. Available from: /pmc/articles/PMC7826706/
51. Della Corte CM, Viscardi G, Papaccio F, Esposito G, Martini G, Ciardiello D, et al. Implication of the Hedgehog pathway in hepatocellular carcinoma. World J Gastroenterol [Internet]. 2017 Jun 6 [cited 2023 Aug 10];23(24):4330. Available from: /pmc/articles/PMC5487497/
52. Jeng KS, Jeng CJ, Jeng WJ, Sheen IS, Li SY, Leu CM, et al. Sonic Hedgehog signaling pathway as a potential target to inhibit the progression of hepatocellular carcinoma. Oncol Lett [Internet]. 2019 [cited 2023 Aug 10];18(5):4377. Available from: /pmc/articles/PMC6781692/
53. Liu S, Guo W, Shi J, Li N, Yu X, Xue J, et al. MicroRNA-135a contributes to the development of portal vein tumor thrombus by promoting metastasis in hepatocellular carcinoma. J Hepatol [Internet]. 2012 Feb [cited 2023 Nov 6];56(2):389–96. Available from: https://pubmed.ncbi.nlm.nih.gov/21888875/
54. Sun JX, Shi J, Li N, Guo WX, Wu MC, Lau WY, et al. Portal vein tumor thrombus is a bottleneck in the treatment of hepatocellular carcinoma. Cancer Biol Med [Internet]. 2016 Dec 1 [cited 2023 Nov 6];13(4):452. Available from: /pmc/articles/PMC5250602/
55. Guo W, Xue J, Shi J, Li N, Shao Y, Yu X, et al. Proteomics analysis of distinct portal vein tumor thrombi in hepatocellular carcinoma patients. J Proteome Res [Internet]. 2010 Aug 6 [cited 2023 Nov 6];9(8):4170–5. Available from: https://pubmed.ncbi.nlm.nih.gov/20583822/

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Original Research

The Impact of Graft Type on the Outcome of Liver Transplantation for Hepatocellular Carcinoma

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Abstract

Objectives: The impact of graft type on the outcomes of liver transplantation (LT) for hepatocellular carcinoma (HCC) remains controversial. We aimed to evaluate the efficacy of living donor (LDLT) versus deceased donor LT (DDLT) by analyzing disease-free survival (DFS) and overall survival (OS).

Methods: We evaluated 356 HCC patients who underwent LT. There were two groups: LDLT and DDLT. We compared OS, DFS, demographic data, and clinical variables between the cohorts. The study also evaluated the impact of Milan criteria adherence on patient outcomes.

Results: The study population was 85.9% male, with a mean age of 52.69 (±0.61) years.While nearly half of the patients were within the Milan criteria, a significant majority of procedures, n=356 (94.7%), were LDLT. The OS and DFS were comparable between the groups, irrespective of Milan criteria compatibility. Patients with tumors within the Milan criteria demonstrated a significantly better OS and DFS compared to those with tumors beyond the criteria ($p<0.001$).

Conclusion: The Milan criteria remain the best prognostic indicator after liver transplantation for HCC. Liver graft type (LDLT vs. DDLT) did not affect liver transplantation (LT) outcomes for HCC, regardless of Milan criteria adherence.

Keywords: Liver transplantation, Living donor, Deceased donor, Hepatocellular cancer, Milan criteria, Extended criteria

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Globally, HCC is a significant health burden, ranking as the sixth most common cancer and the third leading cause of cancer-related mortality. The incidence of HCC has steadily increased over the last decade.[1] Hepatitis B virus and liver cirrhosis are risk factors for HCC. In patients with HCC, there are two diseases: cirrhosis and cancer.[2] Consequently, LT represents the ideal therapeutic approach for the concurrent management of these diseases.[3] The establishment of the Milan criteria had a substantial impact on the treatment of HCC by facilitating the allocation of DDLT to stringently selected patient populations, resulting in favorable long-term survival outcomes. Hence, LT has evolved into the preferred therapeutic modality for HCC. Nevertheless, the Milan criteria are widely regarded as overly restrictive, prompting the development of expanded criteria.The University of California, San Francisco (UCSF) criteria have successfully broadened the Milan criteria by 10% and are presently employed for DDLT organ allocation in patients with HCC within the United States (US).[4]

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On the other hand, in some countries such as Türkiye, Japan, Korea, and India, the deceased donor organ pool is insufficient for the patients waiting for liver transplantation. [5] For this reason LDLT has emerged as a viable alternative for the management of patients with cirrhosis and/or HCC. The effect of graft type on DFS and OS in HCC patients undergoing LDLT is a key area of research in countries where LDLT is prevalent.[6] We aimed to evaluate and compare long-term DFS and OS outcomes following LDLT and DDLT in patients with HCC. We also aimed to evaluate the impact of Milan criteria adherence on the long-term outcomes of patients undergoing LT for HCC.

Methods

Study Design

Over the period from March 2006 to September 2021, 455 patients who received LT for HCC in our Liver Transplant Institute. Recipients whose post-transplant follow-up period is less than 90 days (n=79) were excluded. In total, 376 patients were included for analysis. Three hundred and fifty-six patients underwent LDLT (94.6%) for HCC. The remaining patients underwent DDLT. Preoperative evaluation of all patients included laboratory tests, imaging techniques (Doppler ultrasonography, computed tomographic angiography of the liver, and positron emission tomography scanning) for staging of HCC. In addition, pulmonary and cardiovascular assessments were performed following our pretransplant preparation guidelines.[7] All patients were reviewed by a multidisciplinary tumor board consisting of transplantation surgeons, radiologists, oncologists, and pathologists. Patients were enlisted after receiving approval from the multidisciplinary tumor board. In pre-transplantation treatments, patients underwent radiofrequency ablation, transarterial chemoembolization (TACE), transarterial radioembolization (TARE), and resection. Resection was performed on Child A patients with a single lesion greater than 2 cm, provided that portal venous pressure and bilirubin levels were normal. For patients who were not suitable for resection, including those with Child A and B status, and a performance status of 0-2, bridging or downstaging treatments with TACE or TARE were applied while waiting for a donor.

Data Collection

Data collection, including demographic and clinical variables such as age, gender, liver disease etiology, Model for End-Stage Liver Disease (MELD) score, Child-Pugh classification, preoperative alpha-fetoprotein (AFP) levels, pretransplant locoregional therapies, and histopathological reports, was performed through reviewing the patient medical records.

Follow-Up Period After LT

Serum AFP levels were monitored monthly during the first year following LT. Every three months thereafter. A follow-up schedule of contrast-enhanced chest and dynamic liver computed tomography (CT) scans was implemented, with scans performed every three months for the first two years post-liver transplantation (LT), and every six months thereafter.Throughout the follow-up period, contrast-enhanced liver magnetic resonance imaging (MRI) and positron emission tomography (PET) scans were planned annually. Patients underwent whole-body bone scintigraphy every six months during the initial two years following LT, and annually thereafter. Once locoregional recurrence or distant metastases were detected, treatment modalities such as liver resection, loco-regional therapies, or systemic therapy were administered.

Immunosuppressionprotocol After LT

Prednisolone, calcineurin inhibitors (CNI), and mycophenolate mofetil (MMF) were the primary immunosuppressive agents used for the first postoperative month. Afterwards, MMF was discontinued and mammalian target of rapamycin inhibitors (mTORi) such as everolimus were introduced into the immunosuppressive regimen, while the tacrolimus dose was reduced. Prednisolone was discontinued at the end of the third month following LT.

Statistical Analysis

Sample size was determined through a power analysis by using G-Power 3.1 software (Heinrich-Heine-Universität Düsseldorf, Germany). With a power (1-β) of 0.80 and a significance level (α) of 0.05, the analysis yielded a calculated sample size of 18 participants per group, resulting in a minimum total sample size of 36 participants. Statistical analyses were conducted using Statistical software Package for Social Sciences (SPSS) version 23 (IBM Corp., Armonk, NY, USA). Continuous variables are reported as mean±standard error, and categorical variables as the number and percentage of affected individuals. Disease-free survival (DFS) was measured from the date of liver transplantation (LT) until hepatocellular carcinoma (HCC) recurrence or until the final documented date of no evidence of tumor recurrence on imaging studies. Kaplan-Meier analysis was used to estimate DFS and OS, with any p-value of less than 0.05 defined as statistical significance.

Results

Between March 2006 and September 2021, 376 patients underwent LT for HCC.Three hundred and twenty-three patients were male (85.9%). Average age was 52.69 (±0.61)

years. The etiology of HCC in 299 (79.5%) patients was viral hepatitis. Transarterial chemoembolization (TACE) was the most common pre-transplant bridging therapy, used in 8.5% of patients. Half of the population met the Milan criteria. Additionally, 356 patients (94.6%) underwent LDLT, while there were 20 (5.4%) DDLT procedures (Table 1).

Survival analyses showed no significant differences in OS (p=0.66) or DFS (p=0.413) between LDLT versus DDLT. The results were comparable regardless of the compatibility with the Milan criteria (within [OS; p=0.604, DFS; p=0.276] vs. beyond [OS; p=0.464, DFS; p=0.333]). Nevertheless, wecompared patients meeting and exceeding the Milan criteria and found that OS and DFS were better in tumors within the Milan criteria (p<0.001) (Table 2) (Fig. 1-4b).The OS of the entire cohort in 1, 3-, 5-, and 7- years following LT were 86.5%, 72.4%, 63.1%, and 57.2%, respectively. The DFS rates were 81.6%, 66.5%, 60.8%, 56.9%, respectively (Table 3).

Table 1. Descriptive analysis of demographic and clinicopathological data

Variables	Mean (Std)	Count (%)
Age, years	52.69 (0.61)	
Gender		
Female		53 (14.1)
Male		323 (85.9)
BMI, kg/m^2	26.16 (0.23)	
Viral		299 (79.5)
Alkol		6 (1.6)
Etiology		
Buddchiari		8 (2.1)
Kriptojenik		50 (13.3)
Others		13 (3.5)
Child-Pugh Classification		
A		127 (33.8)
B		166 (44.1)
C		83 (22.1)
GRWR	0.97 (0.03)	
AFP, ng/mL	387.44 (92.46)	
Tumor size, cm	4.23 (0.2)	
Nodul amount	3.22 (0.21)	
Treatment before Liver Transplantation		
Absence		314 (83.5)
TACE		32 (8.5)
Resection		8 (2.1)
RFA		6 (1.6)
Resection+RFA		1 (0.3)
Resection+TACE	1 (0.3)	
TARE+TACE		4 (1.1)
TARE		10 (2.7)
Milan Criteria		
In		188 (50)
Beyond		188 (50)
Donor type		
LDLT		356 (94.7)
DDLT		20 (5.3)
Recurrence		73 (19.4)

BMI: Body mass index; GRWR: Graft to recipient weight ratio; AFP: alpha-fetoprotein; TACE: Transarterial chemoembolization; RFA: Radiofrequency ablation; TARE: Transarterial radioembolization; LDLT: Living Donor Liver Transplantation; DDLT: Deceased Donor Liver Transplantation.

In the current study, patients were categorized based on whether their HCC were within or beyond the Milan criteria, as determined by pre-transplant radiological imaging. Among the 376 patients, 188 were within the Milan criteria, with the remaining patients beyond those criteria. Within the Milan criteria, 176 (93.6%) patients received LDLT and 12 (6.4%) underwent DDLT. In the group beyond the Milan criteria, 180 (95.7%) patients underwent LDLT and 8 (4.3%) received DDLT.

In HCC within the Milan criteria, 7-year OS rates were 74.7% after LDLT and 36.7% after DDLT (p=0.604). Seven-year DFS rates were 74.6% after LDLT and 40.1% after DDLT (p=0.655). Similarly, for patients with HCC beyond the Milan criteria, OS (p=0.464)and DFS (p=0.282) rates after LDLT were comparable to DDLT (Table 3).

Discussion

We analyzed the long-term DFS and OS outcomes of patients with HCC who received LT, stratified by Milan criteria. The impact of graft type on LT outcomes for HCC remains controversial, with conflicting evidence regarding LDLT. Some studies have reported lower OS and DFS rates following LDLT compared to DDLT for HCC patients. One reason for this is that LDLT offers a quicker method for transplanting patients with HCC, thus bypassing the waiting list period. This waiting list period helps in selecting more biologically more favorable tumors that would benefit more from LT. Another contributing factor is the liver regeneration triggered following transplantation of partial liver grafts in LDLT. The liver regeneration process following transplantation may create an environment conducive to accelerated growth of residual tumors, potentially leading to increased recurrence rates.[8-11] However, several studies have reported comparable OS and DFS rates after LDLT and DDLTin patients with HCC, suggesting that graft type does not influence long-term outcomes.[12-17] Conversely, a recent meta-analysis found a higher incidence of tumor recurrence following LDLT compared to DDLT, which has been attributed to surgical technique. The microscopic residual tumors on the retro-

Table 2. Survey analysis

Variables	Mean OS, years (SD)	HR	95%CI	p	Mean DFS, years(SD)	HR	95%CI	p
Beyond milan criteria		0.536		0.464		0.936		0.333
LDLT	6.45 (0.48)		5.5-7.39		7.88 (0.5)		6.9-8.86	
DDLT	5.76 (1.28)		3.25-8.28		6.76 (1.29)		4.22-9.31	
In milan criteria		0.269		0.604		1.187		0.276
LDLT	10.8 (0.49)		9.84-11.76		13.48 (0.27)		12.89-14.06	
DDLT	6.42 (0.97)		4.52-8.32		7.96 (0.72)		6.55-9.37	
Total population		0.194		0.66		0.669		0.413
LDLT	8.74 (0.38)		7.99-9.49		10.95 (0.34)		10.29-11.61	
DDLT	6.12 (0.83)		4.49-7.75		7.5 (0.77)		6.0-9.0	
Total population		32.5		<0.001		67.74		<0.001
In milan criteria	10.69 (0.48)		9.75-11.64		13.41 (0.30)		12.82-14	
Beyond milan criteria	6.48 (0.47)		5.55-7.4		7.95 (0.48)		6.99-8.913	

OS: Overall Survival; DFS: Disease free Survival; SD: standart deviation; HR: Hazard Ratio; CI: Confidence Interval; LDLT: Living Donor Liver Transplantation; DDLT: Deceased Donor Liver Transplantation.

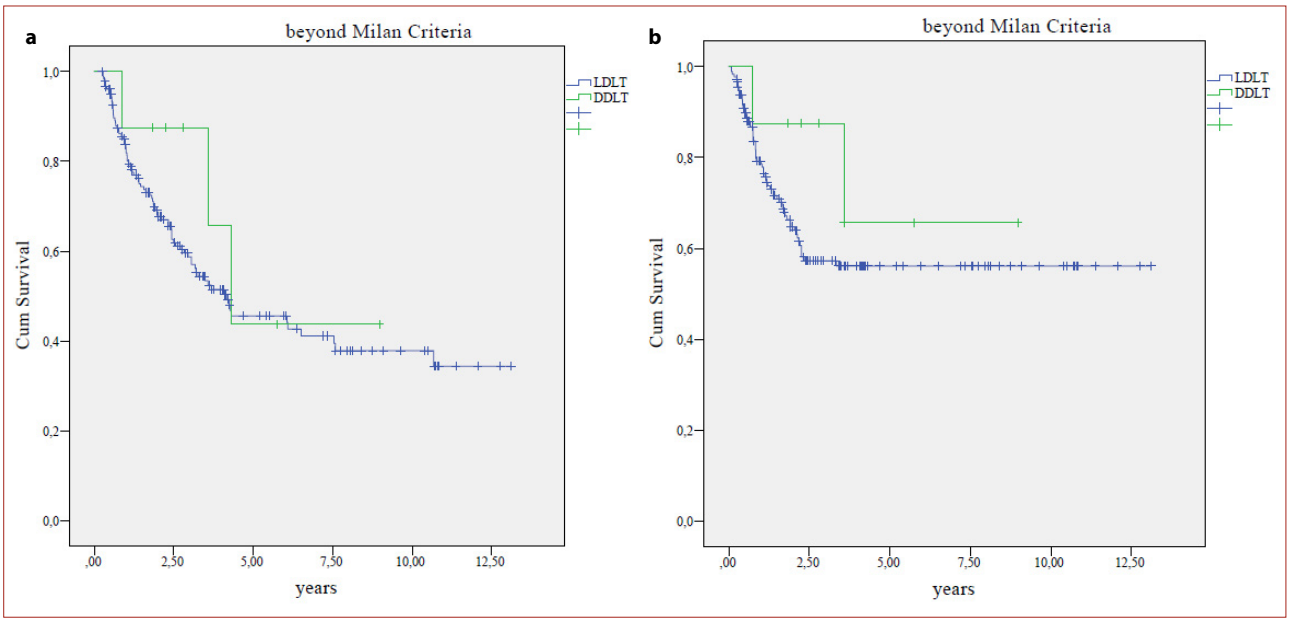

Figure 1. (a) Overall survival analysis of those who underwent LDLT and DDLT in the population beyond the Milan criteria. **(b)** Disease free survival analysis of those who underwent LDLT and DDLT in the population beyond the Milan criteria.

hepatic vena cava and the hepatic vein stumps could have caused the increased recurrence rates observed in the LDLT group.[18]

When evaluating LDLT and DDLT, it is essential to consider the cultural contexts of the countries where the transplant centers are located. In Western societies, DDLTis more common. On the contrary, LDLT is more frequent in Eastern countries such as Japan, Korea, India, and Türkiye.

In HCC, tumors beyond the Milan criteria, survival rates following both DDLT and LDLT progressively decline as tumor size and number increase.[19-22] Similar to prior research, this study demonstrated higher OS and DFS rates in patients within the Milan criteria following both graft types. Specifically, in patients within the Milan criteria, the 7-year OS rate was 73% after both graft types, while in patients beyond the Milan criteria, the 7-year OS rate was 41%. The 7-year DFS rate for patients within the Milan criteria was 73.1%, compared to 40% for those that exceeded Milan criteria.

The long-term DFS and OS rates between patients who un-

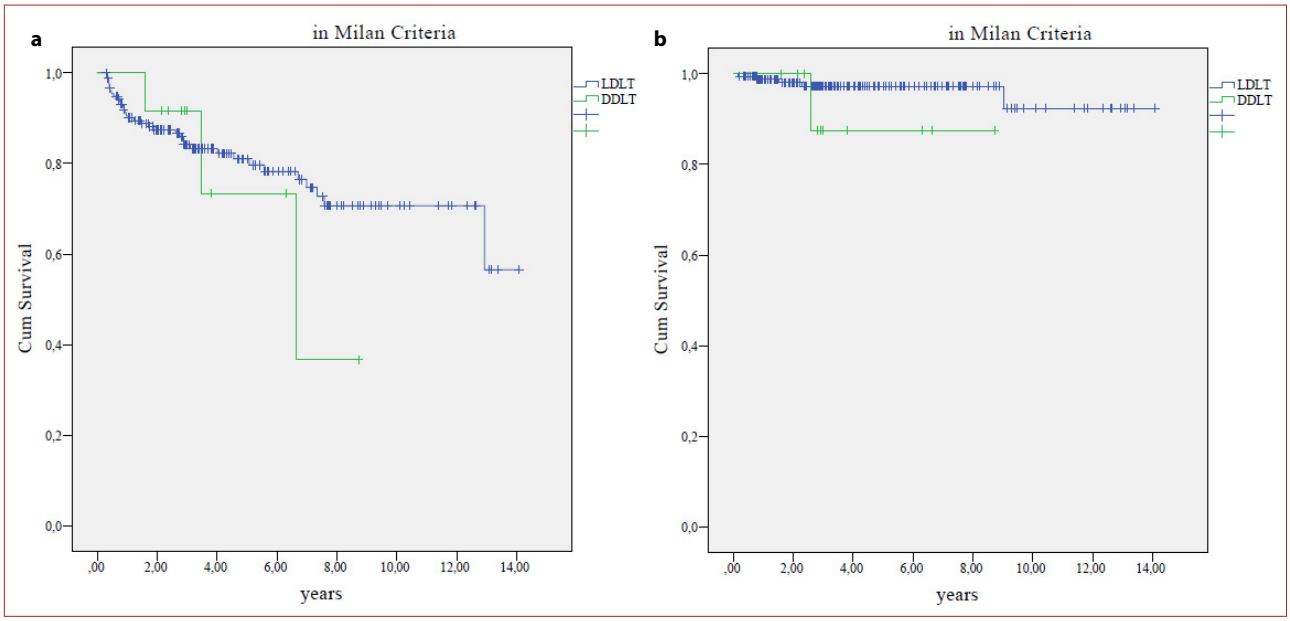

Figure 2. (a) Overall survival analysis of those who underwent LDLT and DDLT in the Milan Criteria. **(b)** Disease free survival analysis of those who underwent LDLT and DDLT in the Milan Criteria.

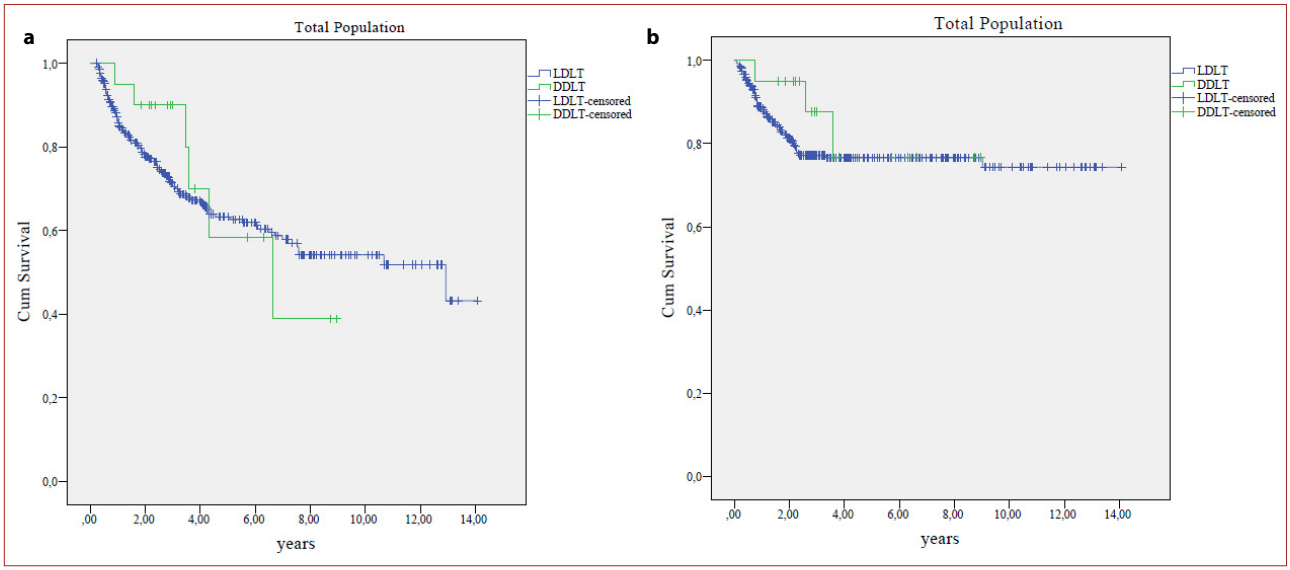

Figure 3. (a) Overall survival analysis of those who underwent LDLT and DDLT in the total population. **(b)** Disease free survival analysis of those who underwent LDLT and DDLT in the total population.

derwent LDLT or DDLT for HCC were comparable, regardless of their compatibility with the Milan criteria. Regardless of compatibility with the Milan criteria, the 7-year OS rates following LDLT were 57.9%, compared to 38.9% following DDLT. Although the 7-year DFS rate was higher in the LDLT group (57.3%) compared to the DDLT group (41.5%), this difference did not reach statistical significance. Our study demonstrated that graft type did not affect tumor recurrence or patient survival rates. Furthermore, patients within the Milan criteria exhibited superior 7-year OS and DFS rates following LDLT compared to DDLT. We hypothesize that the observed lower recurrence rates in the LDLT group may be attributed to a mean graft recipient weight ratio (GRWR) exceeding 0.8.[13]

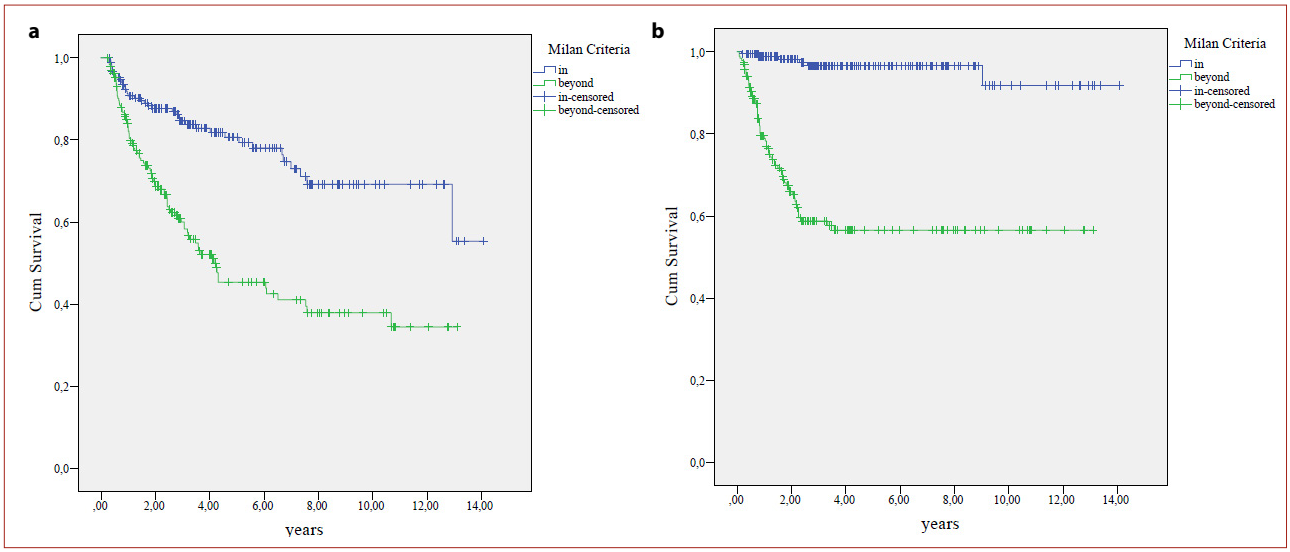

Figure 4. (a) Overall survival analysis of the groups within and beyond the Milan Criteria in the population. **(b)** Disease free survival analysis of the groups within and beyond the Milan Criteria in the population.

Table 3. OS and DFS rates of patients in the study population

	OS (%)					DFS (%)				
Variables	**1 year**	**3 years**	**5 years**	**7 years**	**p**	**1 year**	**3 years**	**5 years**	**7 years**	**p**
All Cohort, (n: 376)	86.5	72.4	63.1	57.2	-	81.6	66.5	60.8	56.9	-
In Milan DDLT, (n:12)	100	91.7	77.3	36.7	0.604	100	80.2	80.2	40.1	0.655
In Milan LDLT, (n:176)	90.1	84.2	81.0	74.7		89.5	82.8	79.6	74.6	
Beyond Milan DDLT, (n:8)	87.5	87.5	43.8	43.8	0.464	87.5	87.5	43.8	43.8	0.282
Beyond Milan LDLT, (n:180)	83.1	58.7	45.6	41.1		73	48.3	41.4	39.8	
In Milan (DDLT&LDLT) (n:188)	90.7	84.6	80.7	73	<0.001	90.2	82.6	79.6	73.1	<0.001
Beyond Milan (DDLT&LDLT) (n: 188)	82.1	60.0	45.3	41		73.0	50.1	41.5	40	
LDLT (In and beyond Milan) (n:356)	86.6	71.4	63.3	57.9	0.660	81.2	65.4	60.6	57.3	0.411
DDLT (In and beyond Milan) (n:20)	95	90	58.3	38.9		95	83.1	62.3	41.5	

OS: Overall survival; DFS: Disease free survival; DDLT: Deceased donor liver transplantation; LDLT: Living donor liver transplantation.

In the present study, the majority of LT for HCC was LDLT. In Türkiye and many other Asia countries, deceased donors are scarce, so LT programs mainly perform LDLT. For this reason,LDLT has been the main therapeutic approach for patients with HCC in these countries.[23-24] Advantages of LDLT include eliminating the waiting list duration, thus reducing the risk of recurrence. It also provides optimal grafts for patients, contributing to enhanced recovery in the postoperative period. On the other hand, morbidity in the living donor as a result of graft procurement, as well as postoperative complications such as biliary leaks and subsequent sepsis, remains a disadvantage.

The primary limitation of this study is its single-center, retrospective design. Furthermore, the disparity in patient numbers between groups, resulting from constraints in cadaveric donor availability and subsequent limitations in DDLT is acknowledged. Multicenter studies would mitigate this imbalance and enhance future investigations in this field.

Conclusion

Our study demonstrated comparable long-term outcomes between DDLT and LDLT for HCC, regardless of Milan criteria adherence. However, it should be noted that LDLT must be performed within ethical guidelines, as it may lead to undesirable morbidities in the donor. It is also important to acknowledge that patients exceeding the Milan criteria had significantly poorer prognoses compared to those meeting the criteria, irrespective of whether they underwent LDLT or DDLT.

Disclosures

Ethics Committee Approval: This study has been approved by Inonu University Institutional Review Board (Approval no: 2022/3730, date: 26-07-2022).

Conflict of Interest: None declared.

Financial Disclosure: None.

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References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-263.
2. Audureau E, Carrat F, Layese R, Cagnot C, Asselah T, Guyader D, et al. Personalized surveillance for hepatocellular carcinoma in cirrhosis - using machine learning adapted to HCV status. J Hepatol. 2020;73(6):1434-1445.
3. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging,and management of hepatocellular carcinoma: 2018 practice guidanceby the American Association for the Study of Liver Diseases.Hepatology. 2018; 68:723e50.
4. Patel SS, Arrington AK, McKenzie S, Mailey B, Ding M, Lee W, et al. Milan Criteria and UCSF Criteria: A Preliminary Comparative Study of Liver Transplantation Outcomes in the United States. Int J Hepatol. 2012;2012:253517.
5. Rudge C, Matesanz R, Delmonico FL, Chapman J. International practices of organ donation. Br J Anaesth. 2012;108:48-55.
6. Akamatsu N, KokudoN.Liver transplantation for hepatocellular carcinoma from living-donor vs. deceased donor. HepatobiliarySurgNutr. 2016;5(5):422-28.
7. Ince V, Usta S, Carr B, Kutlu R, Dikilitas M, Harputluoglu M, et al. Liver Transplantation for Hepatocellular Carcinoma with Expanded Criteria: Malatya Experience. J Inonu Liver Transpl Inst 2024;2(2):72–77.
8. Lo CM, Fan ST, Liu CL, Chan SC, Ng IO, Wong J. Livingdonor versus deceased donor liver transplantation for early irresectable hepatocellular carcinoma. Br J Surg. 2007;94:78-86.
9. Vakili K, Pomposelli JJ, Cheah YL, Akoad M, Lewis WD,Khettry U, et al.Living donor liver transplantation for hepatocellularcarcinoma: increased recurrence butimproved survival. Liver Transpl.2009;15:1861-1866.
10. Park MS, Lee KW, Suh SW, You T, Choi YR, Kim H, et al. Living-donor livertransplantation associated with higher incidence of hepatocellularcarcinoma recurrence than deceased-donor liver transplantation.Transplantation. 2014;97:71e7.
11. Fisher RA, Kulik LM, Freise CE, Lok AS, Shearon TH,Brown RS Jr, et al.; for A2ALL Study Group. Hepatocellularcarcinoma recurrence and death following livingand deceased donor liver transplantation. Am J Transplant. 2007;7:1601-1608.
12. Lee S, Song GW, Kim KW, Kwon JH, Lee SG. Living Donor Liver Transplantation Versus Deceased Donor Liver Transplantation for Hepatocellular Carcinoma Within or Beyond the Milan Criteria: Comparable Long-Term Outcomes. Transplant Proc. 2021;53(1):92-97.
13. Sandhu L, Sandroussi C, Guba M, Selzner M, Ghanekar A, Cattral MS, et al. Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: comparable survival and recurrence. Liver Transpl. 2012;18(3):315-22.
14. Xiao GQ, Song JL, Shen S, Yang JY, Yan LN. Living donor liver transplantation does not increase tumor recurrence of hepatocellular carcinoma compared to deceased donor transplantation. World J Gastroenterol. 2014;20(31):10953-9.
15. Lei J, Yan L, Wang W. Comparison of the outcomes of patients who underwent deceased-donor or living-donor liver transplantation after successful downstaging therapy. Eur J Gastroenterol-Hepatol. 2013;25(11):1340-6.
16. Ninomiya M, Shirabe K, Facciuto ME, Schwartz ME, Florman SS, Yoshizumi T, et al.Comparative study of living and deceased donor liver transplantation as a treatment for hepatocellular carcinoma.J Am Coll Surg. 2015;220(3):297-304.
17. Liang W, Wu L, Ling X, Schroder PM, Ju W, Wang D, et al. Living donor liver transplantation versus deceased donor liver transplantation for hepatocellular carcinoma: a meta-analysis. Liver Transpl. 2012;18(10):1226-36.
18. Zhang HM, Shi YX, Sun LY, Zhu ZJ. Hepatocellular carcinoma recurrence in living and deceased donor liver transplantation: a systematic review and meta-analysis. Chin Med J (Engl). 2019;132(13):1599-1609.
19. Herrero JI, Sangro B, Quiroga J, Pardo F, Herraiz M, Cienfuegos JA, et al.Influence of tumor characteristics on the outcome of liver transplantation among patients with liver cirrhosis and hepatocellular carcinoma.LiverTranspl. 2001; 7(7):631-6.
20. Roayaie S, Frischer JS, Emre SH,Fishbein TM,Sheiner PA, Sung M, et al. Long-term results with multimodal adjuvant therapy and liver transplantation for the treatment of hepatocellular carcinomas larger than 5 centimeters. Ann Surg. 2002;235(4):533-9.
21. Silva M, Moya A, Berenguer M, Sanjuan F, Andujar RL, Pareja E, et al. Expanded criteria for liver transplantation in patients with cirrhosis and hepatocellular carcinoma.Liver Transpl. 2008;14(10):1449-60.
22. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis.LancetOncol. 2009;10:35–43.
23. Doğan SM, KutlutürkK.Living Donor versus Deceased Donor Liver Transplantation for HCC. J Gastrointest Cancer. 2020;51(4):1104-1106.
24. Isik B, Ince V, Karabulut K, Kayaalp C, Yilmaz S.Living Donor Liver Transplantation for Hepatocellular Carcinoma.Transplant Proc. 2012;44(6):1713-6.

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Original Research

Investigation of Antiproliferative Effect of Phenethyl Isothiocyanate on High-Grade Hepatocellular Carcinoma *in vitro*

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Abstract

Objectives: Hepatocellular carcinoma (HCC) is a primary liver tumor that exhibits significant resistance to conventional chemotherapeutic treatments. Phenethyl isothiocyanate (PEITC), a phytochemical derived from Cruciferae plants, has emerged as a promising therapeutic candidate. We aimed to assess the effects of PEITC on cell proliferation and migration in the SNU-449 HCC cells.

Methods: To evaluate the effect of PEITC (2.5-100 µM) on cell viability and migratory rate in SNU-449 cells, MTT, Sulforhodamine B (SRB), colony formation and wound healing assays were analyzed. All data are presented as the median and interquartile range (IQR).

Results: The results indicated that cell viability in response to PEITC began to decrease at 10 µM after 72 hours, with absorbance values of 0.431 (IQR: 0.458) for the 10 µM treatment and 0.67 (IQR: 0.049) for the control group, respectively ($p<0.05$). The SRB assay results for PEITC-treated and control groups were 0.581 (IQR: 0.789) and 0.381 (IQR: 0.365), respectively ($p>0.05$). The surviving fraction of cells treated with PEITC was 19.35% relative to untreated controls. Wound closure percentages in the PEITC and control groups were 10.35% (IQR: 10.3) and 59.75% (IQR: 15.4), respectively ($p<0.05$).

Conclusion: Our results suggest that PEITC may be beneficial in ameliorating hepatocellular carcinoma. Further comprehensive studies are planned to elucidate the molecular mechanisms underlying the antitumoral effect of PEITC.

Keywords: Hepatocellular Carcinoma, Phenethyl isothiocyanate, antiproliferative, SNU-449 HCC cell line

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Hepatocellular carcinoma (HCC) is recognized as a significant global health issue, particularly in regions with a high diffusiveness of chronic hepatitis B and C infections.[1] The mortality rates associated with HCC are alarmingly high.[2] WHO predicts that liver cancer will cause over a million deaths by 2030.[3] HCC is the most prevalent form of primary liver cancer, responsible for approximately 90% of all cases.[4] Current treatment approaches for HCC include surgery, liver transplantation, immunotherapy, chemotherapy, radiotherapy, and targeted molecular therapies.[5,6] However, challenges such as limited effective treatment options in advanced stages, HCC heterogeneity, and resistance to conventional therapies remain. This necessitates the exploration of new and targeted therapeutic strategies to improve patient outcomes and survival rates.[7] Due to the involvement of various cells and critical factors in the

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development and resistance of HCC treatment, the use of drugs in combination with therapeutic agents or adjuvants is considered an interesting approach to reduce treatment resistance.[8] Phytochemicals, which are naturally occurring compounds derived from plants, have attracted significant interest due to their potential antitumor effects. These compounds mediate their effects through multiple mechanisms, including anti-proliferative, anti-angiogenic, and immunomodulatory activities.[9] Phytochemicals, valuable bioactive compounds found in numerous vegetables, fruits, and plants, have fascinated the scientific community due to their remarkable biological properties and potential health benefits.[10,11]

Phenethyl isothiocyanate (PEITC) is a naturally occurring compound identified in cruciferous vegetables of the Brassicaceae family.[12] Key compounds in this family include watercress, cabbage, cauliflower, turnip, horseradish, broccoli, and Brussels sprouts.[13,14] PEITC possesses important bioactive properties, such as anti-inflammatory, antioxidant, antimicrobial, and anticancer activities.[15] Among its biological effects, PEITC has garnered particular interest due to its strong chemopreventive and anticancer mechanisms. Research has demonstrated that PEITC exerts anti-cancer effects by modulating key survival and signaling pathways, promoting apoptosis, inducing cell cycle arrest, and influencing the activity of drug-metabolizing enzymes.[16] Numerous studies have demonstrated the role of isothiocyanates (ITCs) in reducing HCC growth and metastatic potential.[17,18] In addition, it has been revealed that ITCs can augment the anticancer efficacy of various confirmed chemotherapeutic agents and have the potential for repurposing in the therapy of non-small cell lung cancer, breast cancer, and gastric cancer.[19-21] Ongoing studies are exploring the inhibitory efficacy of PEITC on the carcinogenic progression of several cancer cell types. However, limited data exist regarding its impact on HCC, and the precise mechanisms by which PEITC modulates HCC activity remain unclear. Emerging evidence suggests that the inhibitory effects of natural compounds, such as PEITC, may differ based on the specific HCC cell subtypes. Previously, anticancer properties of PEITC were demonstrated in HCC cell lines such as SK-Hep1, Huh7.5.1, and HepG2.[22-24] However, information regarding the anticancer effects of PEITC on SNU-449 HCC cells and the underlying mechanisms remains unclear. The current study aims to investigate the antiproliferative and migratory properties of PEITC in SNU-449 HCC cells. Our findings suggest that PEITC can significantly suppress proliferative activity and migratory potential. Our results indicated that PEITC may be a favorable potential remedial agent for HCC.

Methods

Preparation of SNU449 Cell Culture

Augmented with 10% heat-inactivated fetal bovine serum (FBS, Sigma-Aldrich) and 1% Penicillin-Streptomycin-Neomycin (Sigma-Aldrich), Roswell Park Memorial Institute (RPMI) medium-1640 was used for culture of the SNU449 human hepatocellular carcinoma cell line (American Type Tissue Collection ATCC, CRL 2234). The cells were kept in a humidified incubator (at 37°C in 5% CO_2).

Cell Viability Assay

SNU-449 cells were resuspended in the RPMI-1640 medium. They were seeded into 96-well plates at a density of 10,000 cells per well followed by overnight incubation. PEITC (Sigma-Aldrich, Germany) (2.5, 5, 10, 20, 50, and 100 µM) was exposed for 24, 48, and 72 hours in RPMI-1640 medium after incubation. Then, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma-Aldrich, Germany) solution (5 mg/ml in phosphate-buffered saline (PBS)) was added and incubated for 4 hours. After the addition of 100 µL Dimethyl sulfoxide (DMSO, Merck, Germany) the plate was gently shaken. Absorbance was then recorded at 570 nm (Biotek, Synergy H1m).

Sulforhodamine B (SRB) Assay

Cytotoxicity was assessed on the SNU-449 cell lines using SRB assay. PEITC (5, 10, and 20 µM) was exposed to SNU-449 cells, plated at 10,000 cells per well in 96-well plates containing RPMI-1640 medium for 48 hours. Then cells were fixed with ice-cold 50% trichloroacetic acid (TCA) and stained with 0.04% SRB (Sigma-Aldrich, Germany). The SRB-bound dye was solubilized using 10 mM Tris-base, and after the addition of 100 µL DMSO (Merck, Germany) the absorbance was measured at 510 nm.

Colony Formation Assay

SNU-449 cells were seeded into 6-well plates at a density of 1,000 cells per well. After incubation overnight, the cells were exposed to 10 µM PEITC for 48 hours. The medium was replenished with RPMI-1640 every two days for 14 days. Colonies were fastened with a methanol: acetic acid (3:1) mixture and stained with 0.5% crystal violet. Colonies were enumerated using a microscope (Leica, DMi8).

The plate 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 PEITC-treated cells / PE of control cells) × 100

Wound Healing Assay

SNU-449 cells were plated in 60-mm cell culture dishes. They were grown to 80% confluence. A scratch was made in the cell monolayer, and the cells were washed twice with PBS to eliminate any cellular debris. Then 10 µM PEITC was treated for 96 hours. Images of the wound area were taken at predetermined time points using a cell imaging system (Leica, Paula). The wound healing area and percentage of wound closure were quantified.

Statistical Analysis

All statistical analyses were carried out using Statistical Package for the Social Sciences software version 27 (SPSS v.27) (IBM, Armonk, NY, USA). The Shapiro-Wilk test was employed to evaluate the normality of the data. Continuous variables are presented as medians with interquartile ranges (IQR). The colony formation assay was analyzed as described previously. To compare continuous variables across multiple groups, a pairwise Kruskal-Wallis test was performed. A p-value below 0.05 was regarded as statistically significant.

Results

The MTT assay demonstrated that the antitumor effect of PEITC was evident starting at a concentration of 10 µM after 72 hours. The absorbance values for the 10 µM and control groups were 0.431 (IQR: 0.458) and 0.67 (IQR: 0.049), respectively ($p<0.05$) (Fig. 1). Given that this represented the lowest effective dose of PEITC, this concentration was selected for subsequent experimental procedures.

We analysed the effects of PEITC 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 PEITC doses and the untreated control group (0.581 [IQR: 0.789] vs. 0.381 [IQR: 0.365]; $p>0.05$) (Fig. 2).

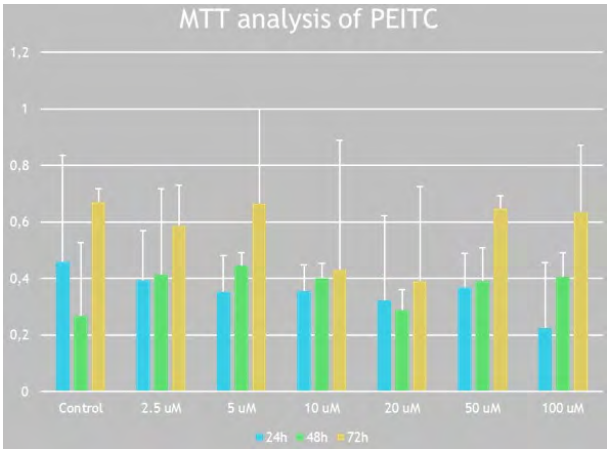

Figure 1. Efficacy of PEITC on cell viability of SNU449 HCC cells.

The migratory characteristics of SNU449 cells were determined via a wound healing assay after 96 hours of 10 µM PEITC treatment. The wound areas of PEITC-treated cells and the control group were 1,982,061 (IQR: 269,014) μm^2 and 528,861 (IQR: 523,150) μm^2, respectively ($p<0.05$) (Fig. 3).Wound closure rate was 10.35% (IQR:10.3) in PEITC-treated cells after 96 hours. The wound closure rate in the control groups was 59.75% (IQR:15.4) ($p<0.05$) (Fig. 4). The figure 5 shows the changes in the streak width of PEITC and control groups.

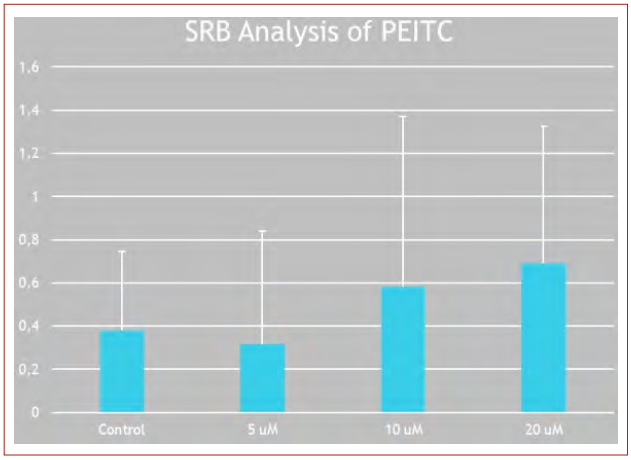

Figure 2. Summary of the results of the SRB assay.

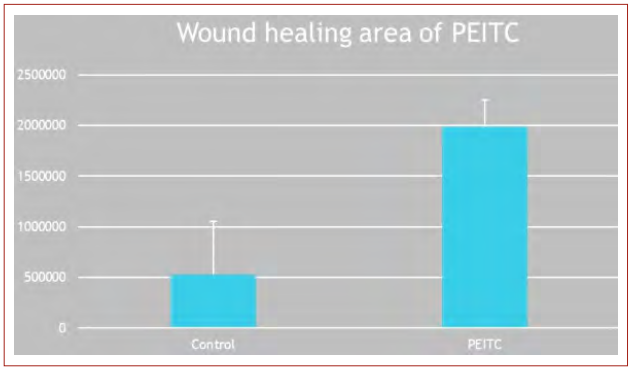

Figure 3. Wound surface area is shown.

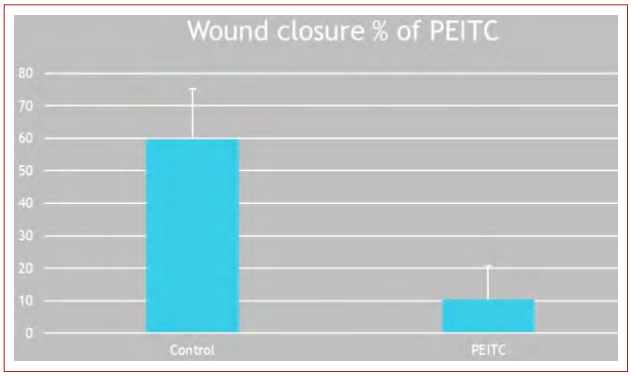

Figure 4. Wound closure rates are summarized.

The colony formation ability of SNU449 cells treated with PEITC was assessed using a colony formation assay. Plate efficiency and survival fraction following PEITC treatment were determined based on the number of colonies formed (Fig. 6). The survival fraction in the colony formation assay of PEITC-treated cells was 19.35% relative to that of untreated cells.

Discussion

Epidemiological research has supported that the consumption of cruciferous plants in the diet assists in reducing the likelihood of malign tumours and that this effect is primarily ascribed to isothiocyanates (ITCs), which are hydrolysis products of glucosinolates.[25] One of the most studied ITCs, PEITC, has been broadly explored for its effects on cancer cells both in vitro and in vivo. The molecular targets of PEITC encompass several hallmark features of cancer, such as apoptosis, proliferation, invasion, and metastasis. A significant advantage of PEITC is its selective action against cancer cells compared to normal cells.[26] This characteristic makes it an attractive candidate for clinical application with minimal side effects. PEITC has been shown to be selectively cytotoxic against various cancer cell types.[27]

In the current research, we examined the effects of PEITC on cell viability, colony formation ability, and migration capacity in SNU449 liver carcinoma cells. Our findings suggest that PEITC can impact various cancer pathways, including those involved in cell survival, migration, and colony formation. These results show that while PEITC reduces specific cancer-related markers, it also induces apoptosis. Additionally, using a wound healing assay for cell migration, PEITC significantly suppressed the migration of SNU449 cells. Exposure to PEITC strongly reduced the survival of cell lines in a dose-dependent way. Cell viability was remarkably reduced in a concentration—and time-dependent way for each PEITC exposure, with more significant cytotoxic effects observed. Research by Crowley E et al. investigated the anticancer effects of natural and synthetic ITC derivatives. The results showed that all ITC derivatives reduced cell viability in a dose- and time-related manner in HepG2, DU145, and 22Rv1 cell lines. The researchers demonstrated that the enhancement exposure period resulted in a more pronounced cytotoxic effect in all cell lines studied. In this study, PEITC, which had the longest carbon chain length, significantly decreased cell viability after exposure to higher concentrations in all cell lines.[28] The carbon chain length affects the chemopreventive activity.[29] The chemical features of PEITC allow it to remain stable in the cell microenvironment and be effectively absorbed and used by liver cells, as a result of that, augmenting its goal assault on HCC cells.[30] Therefore, PEITC holds notable potential for the treatment of HCC. However, the complete therapeutic effects and underlying

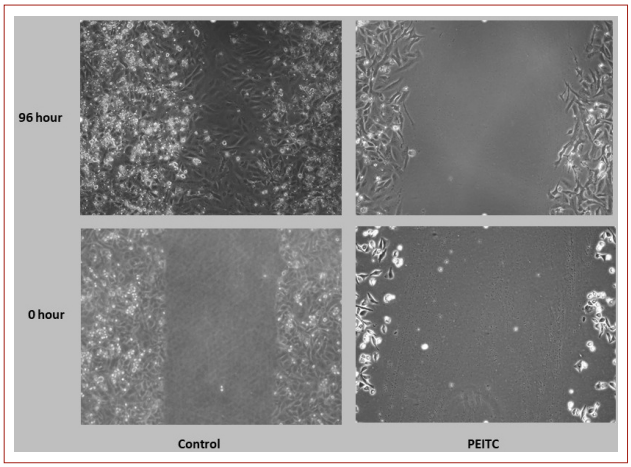

Figure 5. Migratory characteristics of SNU449 cells control and PEITC-treated were evaluated by wound healing assay. It is presented the changes in the streak width of the study groups.

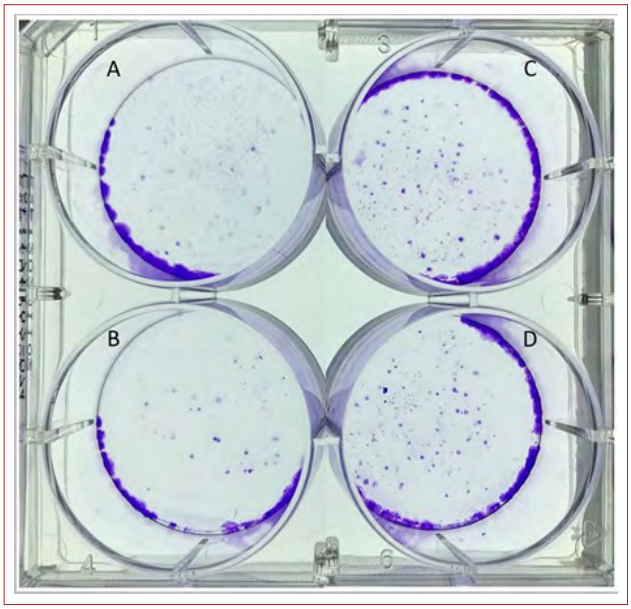

Figure 6. The results of the colony formation assay are summarized. Proliferative potency of SNU449 cells with the PEITC-treated and control group was shown.

mechanisms of PEITC on HCC have not yet been fully elucidated. Rose P et al. found that PEITC inhibited DNA synthesis in HepG2 cells. The G2/M phase of the cell cycle was arrested, which was associated with changes in the protein levels of cyclin B1 and p34cdc2 by PEITC. These results indicate that PEITC effectively triggered apoptosis and cell cycle arrest in HepG2 cells.[22] Another study conducted on HepG2 cells demonstrated that PEITC could stimulate ROS formation and impact microtubule depolymerization, resulting in apoptotic and necrotic cancer cell death.[31]

The induction of apoptosis through ROS production in carcinoma cells, as revealed by PEITC, may explain some of the molecular mechanisms underlying its anticancer potency in various cancers, including liver cancer. PEITC treatment produces ROS and initiates both intrinsic and extrinsic apoptotic pathways, eventually leading to caspase-3 activation.[32] Both previous studies and the current investigation have demonstrated that PEITC exhibits anti-proliferative, anti-migratory, and pro-apoptotic effects in HCC cells.

Translating promising results from in vitro studies to clinical settings remains a considerable challenge. Future investigations must focus on the in vivo therapeutic potential and safety of PEITC using preclinical animal models, alongside the initiation of early-phase human clinical trials to evaluate its efficacy and safety in human subjects. Moreover, given the limitations associated with PEITC's bioavailability and metabolic dynamics, exploring the improvement of innovative nano-formulated drug delivery systems could serve as a crucial direction for advancing the treatment of HCC.

One of the limitations of our study is that it is an in vitro study. While in vitro studies serve as a crucial preclinical step in the evaluation of potential therapeutic agents, they inherently lack the complexity of the tumor microenvironment. Specifically, these models fail to recapitulate the interactions between cancer cells and the surrounding stromal, immune, or vascular constituents, which are critical for tumor progression and response to therapy. As such, in vitro models cannot fully mimic the dynamic conditions of a living organism. To complement these findings, it is imperative to conduct in vivo research using animal models to estimate the efficacy, pharmacokinetics, and toxicity of promising candidates in a physiological context. Another limitation of our study is the absence of mechanistic investigations into the molecular pathways involved in apoptosis. Further investigation is required to elucidate the precise mechanisms by which the treatment induces cell death, thereby enhancing the understanding of its therapeutic potential.

In conclusion, Our study offers promising preliminary data that support the potential use of PEITC in the treatment of HCC. However, further research is necessary in order to have a full understanding of its therapeutic potential and clinical limitations. Through continued investigation, we would like to advance research in the progress of less toxic therapeutic alternatives for HCC, thereby offering patients enhanced treatment options.

Conclusion

The findings from this study indicate that PEITC notably inhibits the viability and migratory potential of liver carcinoma cells. Considering these results, along with evidence from multiple studies, it is reasonable to hypothesize that PEITC may offer therapeutic benefits in slowing the onset or progression of liver carcinoma.

Disclosures

Ethics Committee Approval: None.

Conflict of Interest: None declared.

Financial Disclosure: None.

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References

1. Ozakyol A. Global Epidemiology of Hepatocellular Carcinoma (Hcc Epidemiology). J Gastrointest Cancer. 2017;48:238–240.
2. Konyn P, Ahmed A, Kim D. The Current Trends in the Health Burden of Primary Liver Cancer Across the Globe. Clin Mol Hepatol. 2023;29(2):358–362.
3. Villanueva A. Hepatocellular Carcinoma. N Engl J Med. 2019;380:1450–1462.
4. Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, Finn RS. Hepatocellular carcinoma. Nat Rev Dis Primers. 2021;7:6.
5. Rinaldi L, Vetrano E, Rinaldi B, et al. HCC and Molecular Targeting Therapies: Back to the Future. Biomedicines. 2021;9(10):1345.
6. Vogel A, Bathon M, Saborowski A. Advances in Systemic Therapy for the First-Line Treatment of Unresectable HCC. Expert Rev Anticancer Ther. 2021;21(6):621–628.
7. Lu LC, Hsu CH, Hsu C, Cheng AL. Tumor Heterogeneity in Hepatocellular Carcinoma: Facing the Challenges. Liver Cancer. 2016;5(2):128–138.
8. Tahmasebi Birgani M, Carloni V. Tumor Microenvironment, a Paradigm in Hepatocellular Carcinoma Progression and Therapy. Int J Mol Sci. 2017;18(2):405.
9. Khatoon E, Banik K, Harsha C, et al. Phytochemicals in Cancer Cell Chemosensitization: Current Knowledge and Future Perspectives. Semin Cancer Biol. 2022;80:306–339.
10. Leitzmann C. Characteristics and Health Benefits of Phytochemicals. Complementary Med Res. 2016;23(2):69–74.
11. Ma Z, Zhang H. Phytochemical Constituents, Health Benefits, and Industrial Applications of Grape Seeds: A Mini-Review. Antioxidants. 2017;6(3):71.
12. Wang Y, Wei S, Wang J, Fang Q, Chai Q. Phenethyl isothiocyanate inhibits growth of human chronic myeloid leukemia K562 cells via reactive oxygen species generation and caspases. Mol Med Rep. 2014;10(1):543–549.
13. Palliyaguru DL, Yuan JM, Kensler TW, Fahey JW. Isothiocyanates: Translating the Power of Plants to People. Mol Nutr Food Res.

2018;62(18):1700965.
14. Ishida M, Hara M, Fukino N, Kakizaki T, Morimitsu Y. Glucosinolate Metabolism, Functionality and Breeding for the Improvement of Brassicaceae Vegetables. Breed Sci. 2014;64(1):48–59.
15. Coscueta ER, Sousa AS, Reis CA, Pintado MM. Phenylethyl Isothiocyanate: A Bioactive Agent for Gastrointestinal Health. Molecules. 2022;27(3):794.
16. Ezzat MS, Merghany RM, Abdel Baki MP, Ali Abdelrahim N, Osman SM, Salem MA, et al. Nutritional Sources and Anticancer Potential of Phenethyl Isothiocyanate: Molecular Mechanisms and Therapeutic Insights. Mol Nutr Food Res. 2024;68(8):2400063.
17. Lenzi M, Fimognari C, Hrelia P. Sulforaphane as a Promising Molecule for Fighting Cancer. Cancer Treat Res. 2014;159:207–223.
18. Zhang Y, Huang H, Jin L, Lin S. Anticarcinogenic Effects of Isothiocyanates on Hepatocellular Carcinoma. Int J Mol Sci. 2022;23:13834.
19. Zhang Q, Chen M, Cao L, Ren Y, Guo X, Wu X, Xu K. Phenethyl Isothiocyanate Synergistically Induces Apoptosis with Gefitinib in Non-small Cell Lung Cancer Cells via Endoplasmic Reticulum Stress-Mediated Degradation of Mcl-1. Mol Carcinog. 2020;59:590–603.
20. Kaczyńska A, Herman-Antosiewicz A. Combination of Lapatinib with Isothiocyanates Overcomes Drug Resistance and Inhibits Migration of HER2 Positive Breast Cancer Cells. Breast Cancer. 2017;24:271–280.
21. Yi H, Li Z, Liu X, Dai S, Li S. Therapeutic Mechanism of Lapatinib Combined with Sulforaphane on Gastric Cancer. Evid Based Complement Altern Med. 2021;2021:9933274.
22. Rose P, Whiteman M, Huang SH, et al. β-Phenylethyl Isothiocyanate-Mediated Apoptosis in Hepatoma HepG2 Cells. CMLS, Cell Mol Life Sci. 2003;60:1489–1503.
23. Du J, Zhang Y, Chen J, Jin L, Pan L, Lei P, Lin S. Phenethyl Isothiocyanate Inhibits the Carcinogenic Properties of Hepatocellular Carcinoma Huh7.5.1 Cells by Activating MAPK/PI3K-Akt/p53 Signaling Pathways. PeerJ. 2024;12:e17532.
24. Hwang ES, Lee HJ. Phenylethyl Isothiocyanate and Its N-acetylcysteine Conjugate Suppress the Metastasis of SK-Hep1 Human Hepatoma Cells. J Nutr Biochem. 2006;17(12):837–846.
25. Ngo SN, Williams DB. Protective Effect of Isothiocyanates from Cruciferous Vegetables on Breast Cancer: Epidemiological and Preclinical Perspectives. Anticancer Agents Med Chem. 2021;21(11):1413–1430.
26. Trachootham D, Alexandre J, Huang P. Targeting Cancer Cells by ROS-Mediated Mechanisms: A Radical Therapeutic Approach? Nat Rev Drug Discov. 2009;8:579–591.
27. Gupta P. Phenethyl Isothiocyanate: A Comprehensive Review of Anti-Cancer Mechanisms. Biochim Biophys Acta. 2014;1846(2):405–424.
28. Crowley E, Rowan NJ, Faller D, Friel AM. Natural and Synthetic Isothiocyanates Possess Anticancer Potential Against Liver and Prostate Cancer in Vitro. Anticancer Res. 2019;39(7):3469–3485.
29. Yu R, Mandlekar S, Harvey KJ, Ucker DS, Kong ANT. Chemopreventive Isothiocyanates Induce Apoptosis and Caspase-3-like Protease Activity. Cancer Res. 1998;58:402–408.
30. Yang X, Yang C, Zhang S, Geng H, Zhu AX, Bernards R, Qin W, Fan J, Wang C, Gao Q. Precision Treatment in Advanced Hepatocellular Carcinoma. Cancer Cell. 2024;42:180–197.
31. Pocasap P, Weerapreeyakul N, Thumanu K. Structures of Isothiocyanates Attributed to Reactive Oxygen Species Generation and Microtubule Depolymerization in HepG2 Cells. Biomed Pharmacol. 2018;101:698–709.
32. Wu C, Huang A, Yang J, Liao C, Lu H, Chou S, Chung J, et al. Benzyl Isothiocyanate (BITC) and Phenethyl Isothiocyanate (PEITC)-Mediated Generation of Reactive Oxygen Species Causes Cell Cycle Arrest and Induces Apoptosis via Activation of Caspase-3, Mitochondria Dysfunction and Nitric Oxide (NO) in Human Osteogenic Sarcoma U-2 OS Cells. J Orthop Res. 2011;29(8):1199–1209.

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Original Research

Risk Factors for Early Hepatic Artery Thrombosis After Adult to Adult Living Donor Liver Transplantation

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Abstract

Objectives: Early hepatic artery thrombosis (eHAT) is a primary cause of graft dysfunction, significantly contributing to mortality and morbidity in liver transplant (LT) recipients. This study aims to identify factors influencing the development of eHAT in LT patients at our institution.

Methods: This retrospective study included 428 adult patients who underwent living donor liver transplantation (LDLT) at our institution. The demographic, clinical, and operative characteristics of the remaining 428 patients were analyzed to evaluate risk factors for eHAT development.

Results: eHAT developed in 12 patients (2.8%). The recipient population had a male-to-female ratio of 294:136, a median age of 52 years (range: 18-73), a mean graft-to-recipient weight ratio (GRWR) of 1.06±0.23, and a mean Model for End-Stage Liver Disease (MELD) score of 15.4±6.3. The incidence of eHAT was significantly higher in patients with hepaticojejunostomy (HJ) (5% vs. 2.4%; $p<0.05$) and in those who received cryopreserved artery grafts (CAG) ($p<0.05$). Among the 173 patients (40.2%) with a GRWR <0.98, 9 (5.2%) developed eHAT ($p<0.05$). Furthermore, both erythrocyte suspension (ES) and fresh frozen plasma (FFP) transfusion rates were significantly higher in patients who developed eHAT ($p<0.05$). Preoperative portal vein thrombosis (PVT) was also significantly associated with a higher rate of eHAT ($p<0.05$). Multivariate analysis identified CAG use for arterial reconstruction, HJ for biliary anastomosis, intraoperative FFP transfusion, GRWR <0.98, and t preoperative PVT as independent risk factors for eHAT.

Conclusion: Our findings indicate that hepaticojejunostomy, a graft-to-recipient weight ratio below 0.98, intraoperative FFP transfusion, pre-transplant PVT, and the use of CAG in hepatic arterial reconstruction are significant risk factors for early hepatic artery thrombosis following living donor liver transplantation.

Keywords: Early Hepatic Artery Thrombosis, Living Donor Liver Transplantation, Hepatic Artery Thrombosis, Complications

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A major concern following liver transplantation, hepatic artery thrombosis (HAT) can have devastating consequences. Early hepatic artery thrombosis (eHAT), defined as HAT occurring within the first month, is largely influenced by surgical technique and perioperative factors such as coagulation disorders. Unlike late HAT, which typically arises from immunologically mediated endothelial damage after the first month, eHAT presents with a more acute and severe clinical picture due to the limited collateral circulation in the early post-transplant period. This often leads to graft loss and increased patient mortality if not treated rapidly and accurately. Given the critical nature of eHAT, early diagnosis and treatment are paramount. The reported incidence of eHAT in large studies and meta-analyses ranges from 1.9% to 2.9%.[1-4] Our study aims to investigate the factors associated with eHAT, its management, and the resulting outcomes in adult-to-adult living donor liver transplantation.

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Methods

Evaluation and Selection of Patients for the Study

Between January 2017 and July 2019, we performed 454 living donor liver transplantations (LDLTs) in 452 adult patients. We excluded 24 patients who died within 30 days postoperatively due to causes unrelated to early hepatic artery thrombosis (eHAT). Additionally, the initial transplants of two patients who experienced primary graft nonfunction requiring early re-transplantation were excluded. Consequently, the study cohort comprised 430 liver transplants in 428 patients. Ten patients received double hepatic artery anastomoses, resulting in a total of 438 hepatic artery anastomoses for evaluation.

Operative Procedure

Donor evaluation at our institution has been previously described in detail.[5] During graft implantation, hepatic vein and portal vein anastomoses were performed, followed by graft reperfusion and then hepatic artery anastomosis. We typically performed duct-to-duct biliary anastomosis when the recipient's common bile duct was patent. Hepaticojejunostomy was chosen when the recipient's common bile duct was unsuitable for anastomosis.

We aimed to preserve the vascular arcade supplying the biliary ducts of the recipients during recipient artery preparation. Therefore, we performed meticulous hilar dissection was performed by the recipient surgeons using an en bloc technique.[6] We prioritized anastomosis to a wide recipient artery, avoiding kinking. Therefore, we preferentially selected the recipient common hepatic artery (CHA) if the graft artery had sufficient length. This involved dissecting the CHA from its origin at the celiac axis, extending distally to the origin of the gastroduodenal artery (GDA). The proper hepatic artery and left gastric artery (LGA) were also dissected away from surrounding perivascular lymphatic channels and lymph nodes. The right hepatic artery (RHA), or a replaced RHA if present, was preserved without dissection on the right side of the hepatoduodenal ligament; only its tributaries were ligated with 6/0 polypropylene suture. This approach aimed to preserve the vascular supply to the recipient biliary ducts originating from the GDA.

When the graft hepatic artery had adequate length, the CHA was transected at the optimal plane after applying bull-dog clamps on the GDA (distal) and celiac axis (proximal) sides. Following removal of endothelial remnants from the anastomosis edge of the CHA, the anastomosis was performed, and the GDA side of the CHA was closed with a running polypropylene suture.

In cases with a short graft artery, we preferred the left hepatic artery (LHA), provided its diameter and arterial flow were sufficient to maintain the vascular arcade of the recipient biliary tree via the gastroduodenal and right hepatic arterial arcs. If the recipient LHA was unsuitable, the recipient RHA was used. For extremely short graft arteries or in recipients with arterial dissection or non-ideal recipient arteries, extra-anatomic reconstructions using the splenic artery, LGA, or cryopreserved arterial interposition grafts were employed.[7, 8]

Two transplant surgeons performed all arterial anastomoses. To begin, the right subcostal paddles of the Thomson retractor were loosened, and the graft was elevated with gauze reinforcement. This maneuver allowed the liver to descend, reducing tension on the hepatic artery and facilitating manipulation. Our anastomosis technique involved a "one stay corner suture".[9] Briefly, bulldog clamps were applied to both the graft and recipient sides of the anastomosis. Interrupted 8/0 polypropylene sutures were used under 8X magnification. The anterior wall was completed first, and a clamp was then placed on the last suture and passed beneath the artery to expose the posterior wall for interrupted suturing. After completion, the bulldog clamps were removed.

Intraoperative Doppler ultrasonography (Doppler USG) was used to assess hepatic artery anastomosis patency. Following Ma et al.'s suggestion of a resistive index (RI) between 0.5 and 0.8,[10] we aimed for this range during intraoperative Doppler examinations. If these criteria were not met or patency was questionable, re-anastomosis was typically performed after evacuating any identified thrombus.

Patient Follow-up

Our standard immunosuppressive regimen consisted of mycophenolate mofetil (initiated on postoperative day 1), tacrolimus (initiated on postoperative day 3), and steroids. Methylprednisolone was used for induction and as part of the maintenance therapy with gradual tapering over six months post-transplantation. Once patients were stabilized in the intensive care unit and concerns for bleeding subsided, low molecular weight heparin was initiated as anticoagulant therapy, followed by acetylsalicylic acid starting on postoperative day 10 after discontinuing the low molecular weight heparin.

Diagnosis and Treatment of eHAT

In our country, the Ministry of Health regulations restrict emergency cadaveric donor allocation for HAT-related graft loss to the first 10 postoperative days. Therefore, early detection and intervention (re-anastomosis vs. re-transplantation) are critical for favorable outcomes in eHAT. For the first 10 postoperative days, vascular anastomoses were monitored daily with Doppler ultrasound examinations. In subsequent weeks, Doppler US examinations were performed whenever liver enzymes were elevated. If Doppler ultrasonography showed no flow or low arterial inflow to

Table 1. The demographic, clinical and operative characteristics of the patients

	eHAT	Non-eHAT	p
Recipient age (years)	45 (30-68)	52 (18-73)	0.273
Recipient gender			
Male (n)	8	284	1.000
Female (n)	4	132	
Donor age	29.9 (20-38)	27 (18-61)	0.537
Donor gender			
Male (n)	5	271	0.125
Female (n)	7	145	
Previous abdominal surgery			
Yes	1	21	0.474
No	11	395	
TARE/TACE			
Yes	0	17	0.477
No	12	399	
Graft type			
Right lobe	11	399	0.407
Left lobe	1	17	
MELD score	15.4±6.5	15.4±6.3	0.981
GWRW	0.92±0.16	1.06±0.23	
≤0.98%	9	163	**0.015**
>0.98%	3	253	
ES transfusion			
Yes	6	77	**0.015**
No	6	339	
TDP transfusion			
Yes	4	13	**0.001**
No	8	403	
Preoperative PVT			
Yes	4	34	**0.016**
No	8	381	
Graft artery number			
1	11	406	0.271
≥1	1	10	
CAG use in artery anastomosis			
Yes	3	7	**0.002**
No	9	409	
Recipient anastomosis artery			
Right hepatic artery	2	156	**0.023**
Common hepatic artery	2	104	
Proper hepatic artery	5	99	
Left hepatic artery	1	44	
Double anastomosis	1	9	
Others*	1	4	
Hepatic artery anastomosis			
Anatomic	11	412	0.133
Extra-anatomic	1	4	
Biliary anastomosis type			
Duct to duct	10	408	**0.029**
Hepaticojejunostomy	2	8	
Hepatic vein anastomosis number			
1	2	56	0.508
>1	10	360	
Total	12	416	

Student T test was used for the statistical analyses. TARE/TACE: Transarterial radioembolization /Transarterial chemoembolization; MELD: Model for End-Stage Liver Disease; GWRW: graft-to-recipient weight ratio; ES: erythrocyte suspension; FFP: Fresh Frozen Plasma; PVT: portal vein thrombosis; CAG: Cryopreserved arterial graft; * Splenic artery (n=1), left gastric artery (n=1), aorta with a cryopreserved artery graft (n=1), proper hepatic artery with cryopreserved artery graft (n=2).

the graft parenchyma, computerized tomography angiography (CTA) was performed to confirm the diagnosis of HAT, defined as the absence of arterial flow on CTA. CTA was also used in patients where Doppler USG was inconclusive, and the condition of the patient was inconclusive. Early postoperative eHAT was managed with thrombectomy and re-anastomosis.

If backflow was observed in the graft artery after taking down the initial anastomosis, indicating an intact intrahepatic arterial system, re-anastomosis was performed. Following re-anastomosis, Doppler ultrasonography was performed to assess patency. If the anastomosis was patent but intrahepatic arterial flow was absent on Doppler USG, re-transplantation was considered a salvage procedure. The same follow-up protocol was applied to patients re-operated for eHAT.

Statistical Analysis

The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Continuous variables are presented as mean±standard deviation (mean±SD), or median and range (minimum-maximum values) where appropriate. Categorical variables are presented as the number of patients and percentage (n, %). Differences between dependent and independent variables were analyzed using the Student t-test. Receiver operating characteristic (ROC) curve analysis was used to determine thresholds for categorical variables to eHAT development. Multivariate logistic regression analysis with a backward elimination method was performed to identify independent risk factors for eHAT. Kaplan-Meier survival analysis and the log-rank test were used to compare survival between groups. A p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS version 22 (IBM, Armonk, NY, United States).

Results

Demographic, Clinical, and Operative Characteristics of the Patients

A total of 438 hepatic artery anastomoses were performed in 428 patients. Early hepatic artery thrombosis (eHAT) developed in 12 patients (2.8%). The recipient population had a male-to-female ratio of 294:136. The median age was 52 years (range: 18-73), the mean graft-to-recipient weight ratio (GRWR) was 1.06±0.23, and the mean Model for End-Stage Liver Disease (MELD) score was 15.4±6.3 (Table 1). The most common indications for transplantation were viral hepatitis (27.8%), cryptogenic liver disease (24.8%), and hepatic malignancy (18.9%) (Table 2).

Among the 22 patients with previous abdominal surgery, five underwent re-transplantation. One re-transplantation occurred 2 days post-initial transplant, another at 18 days, and the remaining three more than 3 months later. The types of prior abdominal surgeries included a laparotomy aborted due to hemodynamic instability 4 days before LDLT, partial hepatectomy for hepatocellular carcinoma (HCC), gastroenterostomy, a 30 cm intestinal resection for mesenteric ischemia, and cyst hydatic operation more than 6 months prior to transplantation. In this group, eHAT developed in one patient (6 days post-transplant) who had a previous liver transplantation 6 months prior. Previous abdominal surgery was not significantly associated with the development of eHAT (p>0.05).

Table 2. Etiologies of the liver disease of the patients in the study.

Etiology	eHAT, n (%)	Total patient, n (%)
Acute Liver Failure	1 (4.5)	22 (5.1)
Viral hepatitis	2 (1.7)	119 (27.8)
Hepatitis B Virus	2 (1.9)	108 (25.2)
Hepatitis C Virus	-	11 (2.6)
Malignancy	-	81 (18.9)
Ethanol	-	18 (4.2)
Echinococcus infection	-	9 (2.1)
Budd–Chiari syndrome	1 (4.2)	24 (5.6)
Primary sclerosing cholangitis	1 (7.1)	14 (3.3)
Metabolic liver diseases	-	13 (3.0)
Cryptogenic cirrhosis	6 (5.7)	106 (24.8)
Autoimmune hepatitis	-	17 (4.0)
Chronic rejection	1 (50.0)	2 (0.5)
Others*	-	3 (0.7)
Total	12 (2.8)	428 (100)

*One Giant hepatic hemangioma, 1 Congenital hepatic fibrosis, 1 Sickle cell anemia.

Factors Affecting the Development of eHAT

Early hepatic artery thrombosis (eHAT) occurred in 12 patients, with 10 cases developing within the first 7 days post-transplantation and the remaining two on postoperative days 21 and 22. The factors associated with eHAT development are summarized in Table 1.

The incidence of eHAT was significantly higher in patients who underwent Roux-en-Y hepaticojejunostomy (HJ) (2 of 10, 20%) compared to those with duct-to-duct anastomosis (10 of 420, 2.4%) (p<0.05). Cryopreserved arterial grafts (CAG) were used in 13 patients (2.3%) for hepatic artery anastomosis, and eHAT occurred in 3 of these cases. The use of CAGs was significantly associated with a higher rate of eHAT (p<0.05). Receiver operating characteristic (ROC) curve analysis indicated that a graft-to-recipient weight ratio (GWRW) below 0.98% was a risk factor for eHAT. Among the 173 patients (40.2%) with a GWRW <0.98%, 9 (5.2%) developed eHAT (p<0.005). Intraoperative erythrocyte suspension (ES) transfusion (6 of 84 patients, 7.1%) and fresh frozen plasma (FFP) transfusion (4 of 17 patients, 23.5%) were significantly more frequent in patients who developed eHAT (p<0.05 for both). Preoperative portal vein thrombosis (PVT) was present in 38 patients, and 4 of these patients developed eHAT, indicating a significantly higher eHAT rate in patients with PVT (p<0.05).

Multivariate analysis identified the use of CAG for arterial reconstruction, HJ for biliary anastomosis, intraoperative FFP transfusion, a GWRW ratio <0.98%, and the presence of preoperative PVT as independent risk factors for the development of eHAT (Table 3).

Table 3. Multivariate Analysis of the risk factors for early hepatic artery thrombosis development

Risk Factors	Sig.	OR.	95% CI	
			Lower	Upper
Intraoperative fresh frozen plasma transfusion	<0.001	39.421	6.276	247.596
Cryopreserved artery graft use in artery anastomosis	<0.001	73.295	9.977	538.436
Preoperative portal vein thrombosis	0.002	12.964	2.504	67.116
Hepaticojejunostomy for biliary reconstruction	0.032	12.616	1.243	128.044
Graft weight to recipient weight ratio <0.98%	0.002	20.798	2.960	146.113

Treatment and Outcomes of Patients with eHAT

The treatment and outcomes of the 12 patients who developed eHAT are summarized in Table 4. Briefly, eight patients (66.7%) died after a median follow-up of 26 days (range: 3-93) post-transplantation. Five of these eight patients (62.5%) underwent intervention for eHAT (re-transplantation in one patient on postoperative day 26, thrombectomy and re-anastomosis in four patients). These five patients had documented patent hepatic artery anastomoses on Doppler ultrasonography prior to death. One additional patient was not operated on due to hemodynamic instability. Two patients in this group died later, on postoperative days 83 and 93, due to sepsis.

One patient who developed eHAT on postoperative day 28 underwent revision with a CAG interposition graft between the recipient CHA and the graft artery. Concurrently, a biliary leak from the HJ anastomosis was repaired. Six days later, re-thrombosis of the arterial anastomosis occurred, deemed unsuitable for surgical revision. Tissue plasminogen activator (TPA) was administered via celiac angiography, and intrahepatic biliomas were drained. TPA therapy was unsuccessful, and follow-up imaging showed hepatic artery occlusion. The patient underwent surgery for colonic perforations on postoperative day 79 and died on postoperative day 83 due to sepsis.

The second patient developed eHAT on postoperative day 2 and underwent thrombectomy and re-anastomosis. On postoperative day 17, the patient underwent revision of the HJ anastomosis for a biliary leak. Hepatic artery anastomosis patency was confirmed by Doppler examination on postoperative day 88. On postoperative day 91, infected Dacron extension grafts were removed, and the patient died two days later due to sepsis.

Two patients with eHAT had concomitant vascular pathologies. One patient with vena cava thrombosis due to Budd-Chiari syndrome underwent caval replacement at the time of transplantation. This patient developed eHAT and thrombosis of the caval replacement graft on postoperative day 1. Following revision of both the hepatic artery and caval anastomoses, the patient died two days post-re-intervention. The hepatic artery anastomosis was not patent after re-intervention. The second patient had preoperative portal vein thrombosis and underwent renoportal anastomosis after thromboendovenectomy was deemed insufficient for adequate portal flow. The patient was re-operated for portal vein thrombosis, with anastomosis of the splenic vein to the renoportal shunt. The arterial and biliary anastomoses were initially intact but were taken down for better exposure during this procedure. eHAT developed one day after this re-operation and was revised with a CAG. This patient died three days after the re-intervention; the hepatic artery anastomosis was patent before death.

Four of the 12 patients (33.3%) with eHAT survived following re-intervention. Three of these four patients (75%) underwent re-transplantation at a median of 3 days post-eHAT diagnosis (range: 2-5) and are currently alive. One patient underwent thrombectomy and re-anastomosis on postoperative day 6 and is alive at 14 months post-transplant without biliary complications but with hepatic artery stenosis.

Kaplan-Meier analysis demonstrated significantly lower survival rates in patients who developed eHAT compared to those who did not ($p<0.001$, Fig. 1).

Discussion

The factors contributing to the development of early hepatic artery thrombosis (eHAT) remain debated. We believe that the length and quality of both the graft artery and the recipient artery selected for anastomosis are crucial for successful hepatic artery anastomosis and eHAT prevention. While we lack objective measurements of arterial length, we infer that sufficient length is necessary to achieve a tension-free anastomosis. Assessing arterial quality is challenging due to the absence of definitive criteria beyond the identification of dissection or mural lesions such as atherosclerotic plaques or thrombi. Consequently, thorough evaluation of the donor hepatic artery prior to procurement is essential. Although multiple hepatic artery anastomoses are not established as a risk factor for eHAT, performing a single anastomosis is technically simpler and

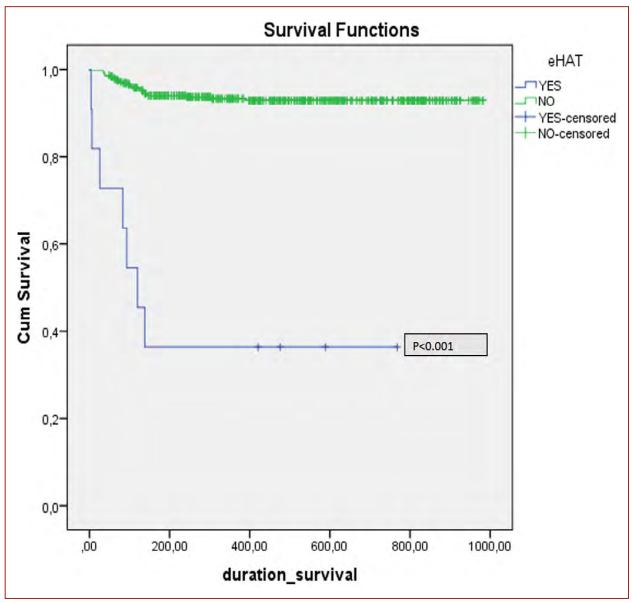

Figure 1. The overall survival of the patients with and without eHAT.

Table 4. The treatment and outcome of the patients with early hepatic artery thrombosis

Case	Age/ Gender	Etiology	MELD score / GWRW	Recipient anastomosis artery	Graft use in artery anastomosis	Biliary reconstruction	Preoperative PVT	Day of eHAT	Treatment	Recipient outcome/ survival
1	22 / M	Chronic rejection	19 /1.03	RHA from SMA	-	HJ	-	28	Thrombectomy and reanastomosis to CHA with CAG, revision of HJ anastomosis for biliary leak. Recurrent eHAT developed on 6 days after reanastomosis. TPA with angiography and drainage of intrahepatic bilioma were performed. POD 79 hemicolectomy and colostomy for colon perforation.	Exitus on POD 83 (While waiting for new graft).
2	41 / M	Budd–Chiari syndrome	13.5 / 0.81	AHP	-	Duct to duct	-	1	Thrombectomy and reanastomosis for eHAT and inferior vena cava graft thrombosis	Exitus on POD 3
3	40 / M	Hepatitis B Virus	13.7 / 0.98	Cryopreserved Y arterial graft between AHP* and graft double artery	Yes	Duct to duct	-	1	Cadaveric retransplantation postoperative 3th day.	Alive (26 months)
4	31 / M	Caroli disease	14.9 / 0.76	AHP	-	Duct to duct	Yes	2	Thrombectomy and reanastomosis for eHAT and renoportal anastomosis thrombosis	Exitus on POD 4
5	54 / F	Cryptogenic cirrhosis	19.2 / 0.79	LHA	-	HJ	-	2	Thrombectomy and reanastomosis to recipient RHA. Biliary leak developed on POD 17th day and HJ anastomosis was revised. On POD 91st day the infected dacron graft was removed and patient died two days after this procedure.	Exitus on POD 93
6	30 / F	Cryptogenic cirrhosis	8.7 /0.8	CHA	Yes	Duct to duct	-	5	Thrombectomy and reanastomosis in postopertative 5th day and cadaveric retransplantation in postopertative 6th day.	Alive (18 months)

Table 4. The treatment and outcome of the patients with early hepatic artery thrombosis (Cont.)

Case	Age/ Gender	Etiology	MELD score / GWRW	Recipient anastomosis artery	Graft use in artery anastomosis	Biliary reconstruction	Preoperative PVT	Day of eHAT	Treatment	Recipient outcome/ survival
7	48 / M	Hepatitis B Virus	14.5 / 1.09	AHP	-	Duct to duct	Yes	21	Revision duct to duct to HJ in postopertative 20th day for biliary leak. eHAT occurred in postopertative 21th day. Enlisted for retransplantation. The hepatic arterial flow was low and intrahepatic bilioma developed.	Exitus on POD 118 (while waiting for new graft).
8	40 / M	Cryptogenic cirrhosis	10 / 0.83	RHA	-	Duct to duct	-	6	Thrombectomy and reanastomosis	Alive (14 months with HA stenosis)
9	48 / F	Cryptogenic cirrhosis	14.2 / 1.32	Splenic artery	-	Duct to duct	Yes	4	Thrombectomy and reanastomosis on POD 4. Recurrent eHAT developed on POD 24 and reanastomosis to recipient celiac artery with CAG. Cadaveric retransplantation performed on POD 25.	Exitus on POD 26
10	68 / M	Cryptogenic cirrhosis	9.2 / 0.81	CAG between AHP** and graft artery	Yes	Duct to duct	-	1	Thrombectomy and reanastomosis POD 1 and cadaveric retransplantation POD 2.	Alive (12 months)
11	67 / M	Cryptogenic cirrhosis	20.2 / 0.91	AHP	-	Duct to duct	Yes	3	No treatment (hemodynamic instability)	Exitus on POD 5
12	45 / F	Acute Liver Failure	31.6 / 0.91	CHA	-	Duct to duct	-	22	No treatment. Enlisted for cadaveric retransplantation.	Exitus on POD 135 (while waiting for new graft)

M: Male; F: Female; MELD: Model for End-Stage Liver Disease; PVT: Portal vein thrombosis; eHAT: early hepatic artery thrombosis; RHA: Right hepatic artery; SMA: Superior mesenteric artery; HJ: Roux-en-Y Hepaticojejunostomy; CHA: Common hepatic artery; CAG: Cryopreserved arterial graft; TPA: Tissue plasminogen activator; POD: Postoperative day; AHP: Arteria hepatica propria; LHA: Left hepatic artery; *In this patient, initially the hepatic artery anastomosis was performed to the recipient RHA. Intraoperatively thrombosis and dissection of the graft artery was observed. CAG was used to reconstruct the anastomosis between recipient AHP and anterior and posterior segmental branches of the graft RHA. Doppler ultrasonography showed a resistant flow pattern in the intrahepatic arterial branches and emergency enlisting was performed. ** Initially, anastomosis was performed to recipient RHA. Intraoperative thrombosis developed and was revised with cryopreserved arterial graft to AHP.

faster.[1] Therefore, when multiple donor candidates are available, selecting a graft with a single artery of adequate length and diameter should be prioritized.[2]

The choice of recipient hepatic artery for anastomosis is vital for successful arterial reconstruction. The length of the anastomosis is important, as excessive length can lead to kinking. At our institution, when the graft artery has sufficient length, we perform hepatic artery anastomosis between the graft artery and the recipient common hepatic artery. However, situations arise where the graft artery is short, the graft has multiple hepatic arteries, or the recipient artery is of poor quality. In these instances, we utilize alternative recipient arteries such as the splenic artery, left gastric artery, or perform anastomosis to the recipient aorta using cryopreserved aortic grafts (CAGs) as interposition conduits. The use of cryopreserved aortic grafts in hepatic artery anastomosis and extra-anatomic anastomoses has been associated with a high incidence of eHAT.[11-14] Consistent with this, our present study identified the use of CAGs in hepatic artery reconstruction as an independent risk factor for eHAT.

The impact of transarterial chemoembolization (TACE) or transarterial radioembolization (TARE) therapy on eHAT remains controversial. These locoregional treatments for hepatocellular carcinoma (HCC) are also commonly used for downstaging HCC beyond Milan criteria. A potential risk of hepatic artery damage exists during catheter placement, either mechanically or due to the administered chemotherapeutic agents. Our institutional experience prior to 2017 showed a significantly higher incidence of eHAT following transarterial procedures.[15] However, in this study (2017-2019), we observed no cases of eHAT after TACE or TARE. This improvement may be attributed to the increased experience of the interventional radiologists and the surgical team's enhanced expertise in hilar dissection.

Preoperative portal vein thrombosis (PVT) was identified as an independent risk factor for eHAT in our study, a finding consistent with the study by Stine et al.[16] While this association may be linked to a tendency towards hypercoagulability, further research is needed to elucidate the underlying mechanisms.

A graft-to-recipient weight ratio (GWRW) of at least 0.8% is generally recommended to prevent small-for-size syndrome (SFSS). However, discrepancies between preoperative volumetric analysis and the actual harvested graft volume can occasionally result in a GWRW below this threshold. Lee et al. have suggested that even a GWRW as low as 0.7% can be safe in selected LDLT cases.[13] Some studies have indicated that a low GWRW or a high recipient-to-donor weight ratio is a risk factor for eHAT.[2, 17-19] Quintini et al. proposed that splenic artery ligation could prevent HAT in recipients with low GWRW,[20] while Kenatkar et al. found no correlation between portal venous pressure or portosystemic gradient and HAT development.[21] In our study, we identified a GWRW cutoff value of 0.98% as an independent risk factor for eHAT, and none of these patients developed SFSS. This finding warrants further investigation.

Hepaticojejunostomy (HJ) as a biliary reconstruction technique was found to be an independent risk factor for eHAT in this study, consistent with our findings in pediatric patients[22] and the study by Baker et al.[23] Baker et al. proposed that this association might be due to potential compression of the Roux-en-Y limb on the arterial anastomosis.

Intraoperative fresh frozen plasma (FFP) transfusion was identified as an independent risk factor for eHAT after LDLT in our study. The first two weeks following LDLT are associated with a hypercoagulable state, during which Protein C and Antithrombin III levels recover.[24, 25] This period, combined with FFP transfusions, may increase the risk of eHAT. Therefore, we recommend avoiding unnecessary transfusions and implementing appropriate anticoagulant therapy during this early postoperative phase. Stahla et al. suggested the use of low-dose heparin (300-400 U/hr) during this period.[24]

Between 2010 and 2012, our institution reported a HAT incidence of 6.5%,[13] during which hepatic artery anastomoses were performed by cardiovascular, orthopedic, and plastic surgeons. Since 2012, transplant surgeons have performed these anastomoses under 8x magnification loupe. We believe that the principles of microsurgery remain consistent regardless of whether a surgical loupe or a microscope is used. Therefore, transplant surgeons experienced in microsurgery can effectively perform hepatic artery anastomosis with an acceptable complication rate.

The present study has some limitations. The first and most important limitation is the fact we could not gşve our data after 2019. This is because we are currently constructing our database and gathering vital information regarding the transplant patients. The second imitation is the fact that we do not have the diameters of opposing arteries which we are currently collectşng for all patients prospectively.

Conclusion

Hepatic arterial anastomosis is a critical step in liver transplantation. Our study has identified the type of biliary reconstruction (HJ), a graft-to-recipient weight ratio below 0.98, intraoperative FFP transfusions, pre-transplant portal vein thrombosis, and the use of cryopreserved arterial grafts in hepatic arterial reconstruction as significant determinants for the development of eHAT. Therefore, comprehensive evaluation of both recipients and donors is of paramount importance for achieving successful arterial reconstruction in living donor liver transplantation.

Disclosures

Ethics Committee Approval: This is a retrospective study.

Conflict of Interest: None declared.

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References

1. Choi HJ, Kim DG, Kim Y, Kwak BJ, Han JH, Hong TH, et al. Clinical course of hepatic artery thrombosis after living donor liver transplantation using the right lobe. Liver Transplant 2018;24(11):1554–60
2. Yang Y, Zhao JC, Yan LN, Ma YK, Huang B, Yuan D, et al. Risk factors associated with early and late HAT after adult liver transplantation. World J Gastroenterol. 2014; 20:10545–52.
3. Piardi T, Lhuaire M, Bruno O, Memeo R, Pessaux P, Kianmanesh R, et al. Vascular complications following liver transplantation: A literature review of advances in 2015. World J Hepatol. 2016; 8:36–57.
4. Bekker J, Ploem S, de Jong KP. Early hepatic artery thrombosis after liver transplantation: a systematic review of the incidence, outcome and risk factors. Am J Transplant 2009; 9: 746- 57.
5. Dirican A, Baskiran A, Dogan M, Ates M, Soyer V, Sarıcı B, et al. Evaluation of potential donors in living donor liver transplantation. Transplant Proc. 2015; 47:1315-8.
6. Abu-Gazala S, Olthoff KM, Goldberg DS, Shaked A, Abt PL. En Bloc Hilar Dissection of the Right Hepatic Artery in Continuity with the Bile Duct: a Technique to Reduce Biliary Complications After Adult Living-Donor Liver Transplantation. J Gastrointest Surg. 2016;20(4):765-71.
7. Yilmaz S, Akbulut S, Kutluturk K, Dogan SM, Baskiran A, Ersan V, et al. Splenic artery transposition for hepatic artery reconstruction during liver transplantation: is it the best choice for adequate arterial inflow in extraordinary conditions? Liver Transpl 2021; 27(4): 595-9.
8. Yilmaz S, Akbulut S, Kutluturk K, Usta S, Koc C, Aydin C, et al. Using the Recipient's Left Gastric Artery for Hepatic Artery Reconstruction in Living Donor Liver Transplantation. Liver Transpl. 2021;27(6): 923-7.
9. Yilmaz S, Kutluturk K, Usta S, Akbulut S. Techniques of hepatic arterial reconstruction in liver transplantation. Langenbeck's Archives of Surgery. 2022;407(7): 2607-18.
10. Ma L, Lu Q, Luo Y. Vascular complications after adult living donor liver transplantation: Evaluation with ultrasonography. World J Gastroenterol. 2016;22(4):1617–26.
11. Stange BJ, Glanemann M, Nuessler NC, Settmacher U, Steinmuller T, Neuhaus P. Hepatic artery thrombosis after adult liver transplantation. Liver Transpl 2003; 9: 612– 20.
12. Miyagi S, Kakizaki Y, Shimizu K, Miyazawa K, Nakanishi W, Hara Y, et al. Arterial and biliary complications after living donor liver transplantation: a single-center retrospective study and literature review. Surg Today. 2018; 48:131–9.
13. Unal B, Gonultas F, Aydin C, Otan E, Kayaalp C, Yilmaz S. Hepatic artery thrombosis-related risk factors after living donor liver transplantation: single-center experience from Turkey. Transplant Proc. 2013; 45:974–7.
14. T. Iida, T. Kaido, S. Yagi, T. Hori, Y. Uchida, K. Jobara, et al. Hepatic arterial complications in adult living donor liver transplant recipients: a single-center experience of 673 cases. Clin Transpl. 2014; 28(9) pp: 1025-30.
15. Ince V, Ersan V, Karakas S, Kutluturk K, Karadag N, Kutlu R, et al. Does preoperative transarterial chemoembolization for hepatocellular carcinoma increase the incidence of hepatic artery thrombosis after living-donor liver transplant? Exp Clin Transplant. 2017; 15:21–4.
16. Stine JG. Pre-transplant portal vein thrombosis is an independent risk factor for graft loss due to hepatic artery thrombosis in liver transplant recipients. HPB. 2015; 18: 279- 86.
17. Lee SD, Kim SH, Kim YK, Lee SA, Park SJ. Graft-to-recipient weight ratio lower to 0.7% is safe without portal pressure modulation in right-lobe living donor liver transplantation with favorable conditions. Hepatobiliary Pancreat Dis Int. 2014;13:18–24.
18. Xue Z, Chen M, Zhang X, Wang G, He X, Wu L, et al. Analysis of early hepatic artery thrombosis after liver transplantation. ANZ J Surg 2018; 88 (03): 172-6.
19. Oh CK, Pelletier SJ, Sawyer RG, Dacus AR, McCullough CS, Pruett TL, et al. Uni- and multi-variate analysis of risk factors for early and late hepatic artery thrombosis after liver transplantation. Transplantation 2001; 71: 767– 72.
20. Quintini C, Hirose K, Hashimoto K, Diago T, Aucejo F, Eghtesad B, et al. 'Splenic artery steal syndrome' is a misnomer: the cause is portal hyperperfusion, not arterial siphon. Liver Transpl 2008; 14(3): 374-9.
21. Kanetkar AV, Balakrishnan D, Sudhindran S, Dhar P, Gopalakrishnan U, Menon R, et al. Is Portal Venous Pressure or Porto-systemic Gradient Really A Harbinger of Poor Outcomes After Living Donor Liver Transplantation? J Clin Exp Hepatol. 2017;7:235-46.
22. Kutlutuk K, Sahin TT, Karakas S, Unal B, Bag HG, Akbulut S, et al. Early Hepatic Artery Thrombosis After Pediatric Living Donor Liver Transplantation. Transplantation proceedings. 2019; 51(4):1162-8.
23. Baker T., Zimmerman MA, Goodrich NP, Samstein B, Pomfret EA, Pomposelli JJ, et al. Biliary reconstructive techniques and associated anatomic variants in adult living donor liver transplantations: The adult-to-adult living donor liver transplantation cohort study experience. Liver Transpl. 2017;23(12):1519-30.
24. Sugawara Y, Kaneko J, Akamatsu N, Imamura H, Kokudo N, Makuuchi M. Anticoagulant therapy against hepatic artery thrombosis in living donor liver transplantation. Transplant Proc. 2002; 34: 3325–26.
25. Stahl RL, Duncan A, Hooks MA, Henderson JM, Millikan WJ, Warren WD. A hypercoagulable state follows orthotopic liver transplantation. Hepatology 1990;12:553–8.

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Original Research

A Network Phenotyping Strategy approach in a Turkish HCC Dataset and Comparison of Patients Selected for Transplant and those who were not

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Abstract

Objectives: A Network Phenotyping Strategy (NPS) was recently created to stage hepatocellular carcinoma (HCC) from an Italian dataset into 25 discrete phenotypes sT, s=1→τ_1, …, s=25→τ_{25} ordered ($\tau_1<\tau_2,\ldots,<\tau_{25}$) according to the dynamics of the HCC progression from its onset.

Methods: To use NPS methodology on an ethnically different, Turkish HCC cohort that had, in addition, been stratified according to patients selected for liver transplantation or not.

Results: The Turkish patients had only a smaller subset of 16 out of the 25 HCC phenotypes of the Italian patients. HCC progression through phenotypes, which are exclusive to the Italian population, is a dominantly tumor biology-driven process, occurring within a constant extent of liver microenvironment damage. In contrast, in phenotypes shared by the minority of Italian patients and the majority of Turkish patients, the HCC progresses by a more complex disease burden generating mechanism, consisting of simultaneous tumor biology-driven damage, accompanied by advancing liver microenvironmental impairment.

NPS objective stratification of a "real world clinical practice" patient cohort into subpopulations with identical HCC clinical phases enabled the simulation of clinician-dependent selection for liver transplantation or not in this cohort, using specific baseline variables. A clearer understanding of HCC biology has allowed us to identify differing biological phenotypes. The predominant 12T phenotype was examined in detail and combined with surgical knowledge obtained retrospectively as to the difference between transplanted and non-transplanted patients to then derive models that may be useful for biology-dependent surgical decision support in future patients.

Conclusion: Only a subset of all HCC phenotypes appeared in the Turkish cohort and may be explained by the non-screened patients having HCC as a secondary problem, and is primarily driven by the liver microenvironment, in contrast to Italy, where HCC develops (predominantly) in healthier livers.

Keywords: HCC, Network phenotyping strategy, transplant

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A Network Phenotyping Strategy (NPS) is a novel non-empirical clinical analytics methodology, explicitly addressing the essential role of disease dynamics in data-driven stage definition, diagnostic and use in prognosis[1-4] through clinically transparent usage of only baseline multi-dimensional clinical data of a real-world patient cohort. We have shown[1,2] that supplementing the patient and visit coherent values of multiple (K) clinical variables by the topology of a complete network of relationships between those values, encoded quantitatively by special K-partite graphs (see Fig. 1) allowed us to characterize the disease progression stage in terms of the personal time to disease onset at the baseline. This unique innovation of NPS addresses the disadvantage of the above cited methods, which do not explicitly include mechanisms for normalizing the patient data change rates according to the biological stage of the disease at baseline.

We recently used a Network Phenotyping Strategy to identify the stages of the disease in patients with hepatocellular carcinoma (HCC) as progression-ordered phenotypes, using an unstratified large Italian database.[2] The purpose of the current study was to use the same NPS methodology on an ethnically different (Turkish) HCC cohort that had also been stratified according to patients who were selected for liver transplantation or not. These Turkish patients differed in having predominantly Hepatitis B (HBV) as a background and minimal or no alcoholism, unlike the Italian patients. Furthermore, they were predominantly diagnosed without a surveillance program and thus, on average, had more advanced disease. We report that in contrast to the Italian patients, the Turkish patients are diagnosed only within a restricted selection of the full spectrum of progression-ordered HCC phenotypes. Quantitative ordering of the NPS diagnosed HCC stages according to their characteristic τ_s allows for studying the τ_s- dependent trends of characteristic clinical variable values without need for longitudinal data.

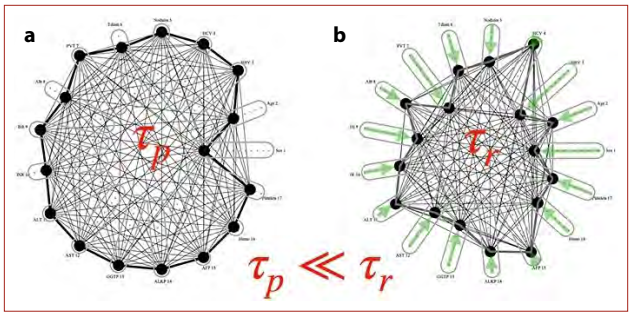

Figure 1. 17-partite graphs $\Gamma_p(\tau_p)$ for two patients with different HCC stages. The 17 ovals represent variables, with internal vertices representing categorical values or sub-intervals of the complete physiological range of respective real-valued variables. Solid circles indicate observed personal baseline values. The network of edges encodes the unique personal clinical context of each variable value. As some specific variable values are typically observed early and other late in disease progression, the topology of the personal relationship network expands or shrinks (see arrows) as the function of baseline time to disease onset. τ_p.

Comparing these trends between the Italian and Turkish patient populations allowed us to hypothesize that observed differences may be due to the HCC in Turkish patients being predominantly driven by the liver microenvironment, in contrast to the more diverse driving factors in European patients. In addition, we used the NPS information about differences between Turkish patients, selected and not selected for liver transplantation at different stages of HCC progression to suggest new insight into the treatment selection and outcomes.

Methods

Patients were characterized by values of 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, 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 [years], gender and HBV/HCV status; B), tumor characteristics, that included maximal diameter (MTD [cm]), tumor uni- or multi-focality, presence/absence of portal vein thrombosis (PVT) and serum α-fetoprotein (AFP [ng/mL]) levels; C), serum liver parameters and blood counts, including levels of albumin [g/dL], total bilirubin [mg/dL], INR, ALT (in relative unit 1/35 [U/L]), AST (in relative unit 1/35 [U/L]), ALKP (in relative unit 1/150 [U/L]), GGT (in relative unit 1/40 [U/L]), Hb [g/dL] and platelets [count/μL].

Ethical Considerations

Database management conformed to legislation on privacy, and this study conforms to the ethical guidelines of the Declaration of Helsinki and approval for this retrospective study on deceased cases and de-identified patients with HCC. This work was approved by the Institutional Ethics Committee (Institutional Review Board Approval No. 2024-6196) for a waiver from obtaining written informed consent for de-identified and mostly deceased patients, in accordance with local guidelines.

Clinical Background of the Analysis

We have two socio-ethnically, institutionally and clinically different populations of patients with the same diagnosed disease (HCC). One (larger, less stratified, surveillance generated) population (ITALICA) provides broad, comprehensive

reference characteristics of HCC progression phenotypes. In the previous study (1), we used the NPS methodology on the surveillance ITALICA cohort, where the broad spectrum of HCC stages is "clinically probed" by individual patient disease statuses. In the other (Turkish), more stratified population, processed by the same NPS algorithm, we expect to see where the real-world "clinical bias" will place these patients in the complete "disease phenotype space" of HCC progression. The goal of this exercise was to obtain better, data-driven personalization of the HCC disease characterization, usable as the decision support information for detailed diagnosis, prognosis or treatment selection. See note 1.

These clinical goals required applying the new, relationship-based NPS method to process the multidimensional (17-variable) characterization of patient clinical profiles. This is because NPS new information, which this method uses to identify the 25 personal HCC progression stages sT, (s=1,…,25) are the networked patient's data relationships, added and quantitatively processed together with conventionally used values of respective clinical variables, collected at the index-visit.

The fact that there are just those 25 progression stages is objectively determined by the NPS processing of the reference ITALICA clinical data relationships in two steps (see ref. 2).

In the first step, the NPS extracts the personal times τ_p from HCC onset from the topologies of observed value relationship networks of every patient.

In the second step, NPS shows that by organizing all patients according to the variability in the stage-dependent presence of early $E_p(\tau_p)$ and late $L_p(\tau_p)$ stage biomarker topologies in those personal networks $\Gamma_p(\tau_p)$, from which τ_p's are computed, the patients emerge naturally grouped into 25 HCC clinically and progression-stage normalized sub-populations, defining the 25 HCC phenotypes, sT. This new NPS-based stage diagnostic of HCC is represented by HCC progression map (see Fig. 2), in which each patient is represented by a τ_p-defined point $[X_p(\tau_p), Y_p(\tau_p)]$, where $X_p(\tau_p)=\log(L_p(\tau_p))$ and $Y_p(\tau_p)=\log(E_p(\tau_p))$. The grouping of all HCC patients into 25 ordered phenotypes emerges naturally from this visualization of this primary result of patient's clinical data processing by NPS.

This new, disease-progression ordered clinical phenotyping information provides 2 practical results:

Firstly, it deconstructs the conventional population averaged data characterizations of the clinical status into the series of 25 objective components, stratified according the progression-ordered sT's.

Secondly, it provides the numerical value of the τ_p-dependent personal clinical burden $CB_p(\tau_p)=Y_p(\tau_p)-X_p(\tau_p)=log\left(\frac{L_p(\tau_p)}{E_p(\tau_p)}\right)$ for every patient. Consequently, once these are aggregated for all patients into their respective 25 HCC progression stages sT, we newly obtained the objective, quantitative, clinically transparent and characteristic values of progression-specific disease burden, $CB_s=\frac{1}{N_s}\sum_{p_s=1}^{N_s}CB_{p_s}$ for all respective progression stages of HCC.

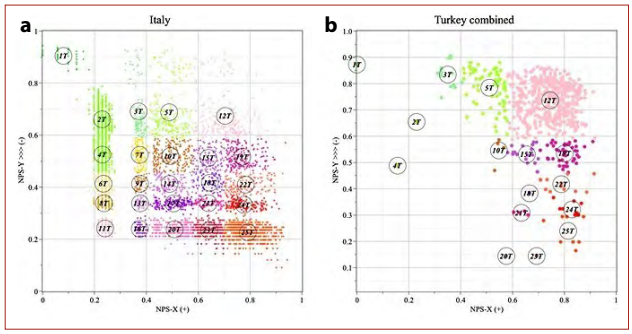

Figure 2. NPS 2-dimensional maps of HCC progression for Italian **(a)** and Turkish **(b)** patients. Each patient is represented as a point. $[X_p(\tau_p), Y_p(\tau_p)]$. X-Y coordinates independently quantify the relative presence of early and late HCC stage networked biomarker topologies in patient's $\Gamma_p(\tau_p)$: 1.0 on the vertical axis and 0.0 on the horizontal axis indicates that we observe 100% of the early stage biomarker network in the patient's graph, while 0.0 on the vertical axis and 1.0 on the horizontal axis indicates that the patient's graph has 100% of the late stage biomarker network. Other values represent personalized combinations of the two prototype topologies and underlying early and late stage biologies sT,(s=1,…,25). The phenotype sub-populations sT,(s=1,…,25) are automatically identified as tight groupings of patients with highly similar clinical profiles: The circles with phenotype labels have centers at the maximum of the exponential distribution of patients in each phenotype region.

Why and how this NPS-Enabled Approach Provides New Clinical Insights

With the exception of higher HBV incidence in Turkish patients and minimal or no alcoholism, other conventional clinical characterization of the 2 populations at the index visit looks very similar, since the frequencies and distributions of the 17 clinical variable values are comparable between the two populations.

The main new clinical information provided by NPS is the de-construction of these single, whole population characteristic values into 25 disease-progression ordered phenotype specific partial averages, computed in sequence by using only data of patients diagnosed with respective HCC progression stages sT. Thus, because we know the characteristic values of $CB_s(\tau_s)$ for each HCC progression stage, we can analyze the clinical HCC progression trends by plotting phenotype-characteristic mean values $\mu_{i,s}=\frac{1}{N_s}\sum_{p_s=1}^{N_s}V$ of respective clinical variables as the function of $CB_s(\tau_s)$.

For supportive computational evaluation of patient's likelihood of being eligible for liver transplant we used multivariate logistic regression regression analysis (JASP software[5]), based upon the baseline data. Starting with all 17 clinical variables, the stepwise, forward, and backward variable selection optimization was performed for patients, identified by NPS analysis in the 12T phenotype (these patients share the same stage of HCC and form 72% of the total population). All optimizations converged to the same best-performing 6-variable model, which includes gender, INR, albumin, hemoglobin, bilirubin, and MTD.

Results

Mean survival of Italian patients is $\mu_{survival}^{Italy}$=1765 days, which is ~1.3 times higher then for Turkish patients $\mu_{survival}^{Turkey}$= 1370 days. Overall mortality (defined as percent of deaths within the 5 year study period) is ~2 times higher in Italy ($\mu_{mortality}^{Italy}$ = 63%) then in Turkish population ($\mu_{mortality}^{Turkey}$ =31%). In Turkish subpopulations, stratified by transplant (T) and non-transplant (*nT*) treatment, the $\mu_{survival}^{TT}$=1626 days, $\mu_{survival}^{T,nT}$=710 days, while mortalities are stratified into $\mu_{mortality}^{TT}$=36% and $\mu_{mortality}^{T,nT}$=19%.

In Figure 2 we compared the complete NPS map of all 25 HCC phenotypes *sT*, (s=1,…,25) from processing screening data for 4802 Italian HCC patients, collected by the ITALICA database (Fig. 2a), and the phenotype assignments of the combined Turkish liver non-transplant (nT) and liver transplant (T) HCC patients (N=681 (N_{nT}=191, N_T= 490)) (Fig. 2b). In this map, each patient is represented by a point, each phenotype is uniquely colored and the circles with phenotype sT labels have the centers at the maximum of the patient distribution in each HCC progression stage phenotype region (see Fig. 3b and c). Progression stages are ordered according to the increasing time to disease onset τ_s. Therefore, the patients in the 1T region of the map are in the earliest diagnosable HCC stage, while patients in the 25T region of the map are in the latest observed HCC stage. Thus, Turkish patients appear in only 16 of the 25 phenotypes seen in the Italian group, with some of those sparsely populated. Specifically, the Turkish patients are not diagnosed with phenotypes *6T, 7T, 8T, 9T, 11T, 13T, 14T, 16T, 17T*, which, in contrast, are heavily populated by Italian patients.

Figure 3 shows in detail the differences between patient distributions across the HCC phenotypes in detail, determined separately for liver transplant (T) and non-transplant (nT) sub-cohorts in the Turkish HCC population. The red and blue distributions above the NPS-X (horizontal) and NPS-Y (vertical) HCC progression axes of the HCC progression stage map (Fig. 3a) show how stage-characterizing networked clinical relationship profiles of individual

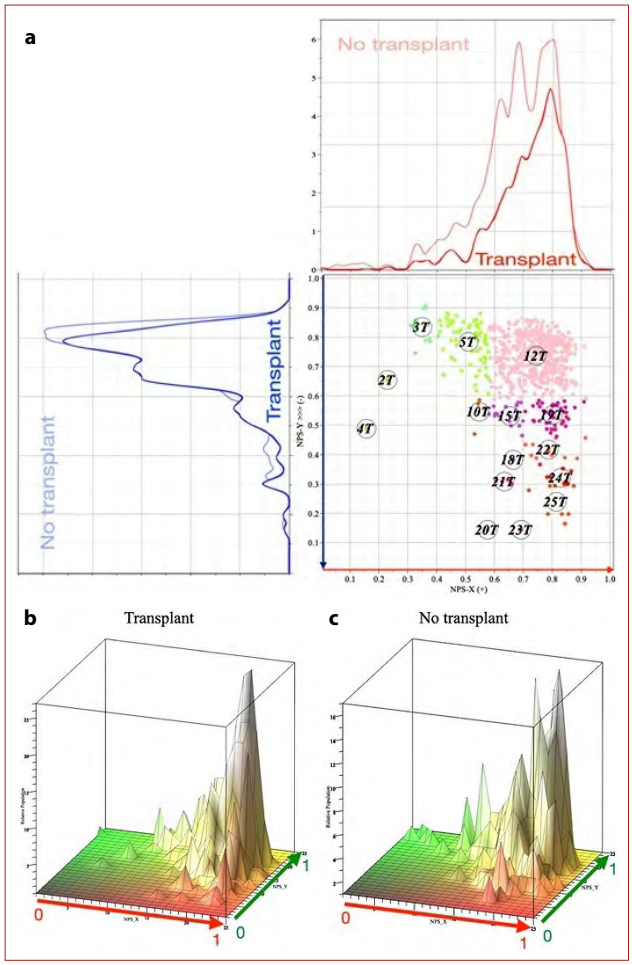

Figure 3. Differences in Turkish patient distribution across HCC phenotypes between transplant and non-transplant subgroups. **(a)** Top and left panels: Projections along the axis capturing the diminishing contribution of earlier stages (blue) and along the late axis capturing the increasing contribution of late stages (red). Light lines: non-transplant, dark lines: transplant. Bottom panels: 3D histograms of patient populations in respective phenotypes for transplant **(b)** and no transplant **(c)** patients.

patients are localized in the two projections of the HCC progression phenotype cases for the T and nT sub-cohorts into characteristics of early HCC stages (Y-axis, blue distributions) and those of later HCC stages (X-axis, red distributions). In the early HCC stages, the levels of NPS early-stage biomarkers in patients's Γ_p (τ_p) are distributed comparably in both treatment-defined groups. In contrast, in the later HCC stages, the late clinical profile biomarker levels in the (smaller) nT patient sub-cohort Γ_p (τ_p)'s span a broader interval than those for the T patients. This corresponds to application of the standard liver transplant selection criteria for these patients. Figure 3b-c show the complete 3D distributions of patients in the LT and nT sub-cohorts. There is

a clear separation of the sub-distributions (peaks) in these 3D distribution plots, delineating the patients with respective HCC stage phenotypes, which, consequently, quantifies the high level of personal clinical similarity of biologies within every patient phenotype sub-population.

We then outlined the NPS-extended experimental characterization of clinical differences between the Italian and Turkish HCC patients, which leads to the elective location of the Turkish cohort in the complete HCC progression phenotype map. For that comparison, we used the transformation of the primary 2-dimensional NPS information about each patient, defined by the coordinate pair $[X_p(\tau_p), Y_p(\tau_p)]$ into one-dimensional, HCC progression phenotype specific clinical burden parameter $CB_p(\tau_p)=Y_p(\tau_p)-X_p(\tau_p)=^{log}\left(\frac{t_p(\tau_p)}{\varepsilon_p(\tau_p)}\right)$. This transformation permitted a study of the trends of 16 phenotype-characteristic mean values $\mu_{i,s}$ of respective clinical variables against the corresponding 16 phenotype-characteristic values of time to disease onset τ_s dependent clinical burdens $CB_s(\tau_s)$.

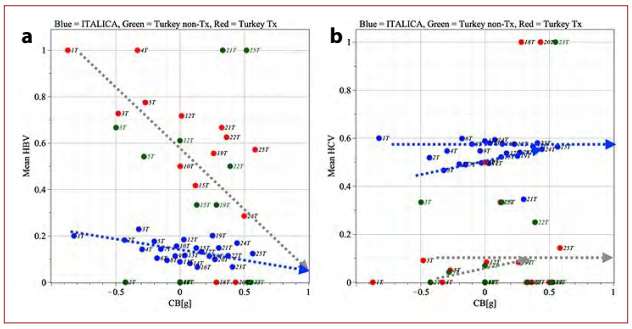

Figure 4. Dependence of phenotype sT-characteristic incidences of HBV **(a)** and HCV **(b)** (vertical axes) on the phenotype characteristic, τ_s-dependent clinical burden $CB_s(\tau_s)$ (horizontal axes) - see Methods for $CB_s(\tau_s)$ definition. Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

In the Turkish HCC population, relatively more males were found in T patients, and with few exceptions, nT patients in all sT phenotypes were slightly older than T patients. On average, Turkey patients were 1.3 younger than Italy patients (μ_{age}^{Italy}=67.5, μ_{age}^{Turkey}=53.5, for Turkey $\mu_{age}^{T,T}$=53.7, $\mu_{age}^{T,nT}$= 52.8).

In the following figures, the data for Italian patients are shown by blue points, and comparisons between T and nT Turkish treatment-based sub-groups for stage-characteristic values of respective clinical variables in their HCC patients is shown by green (nT) and red (LT) points, representing the paired values $[CB_s(\tau_s), \mu_{i,s}^{T,X}]$.

In Figure 4a the higher incidence of HBV cases in the Turkish compared to the Italian patients is shown by the systematic vertical offset of the two trends (gray arrow for Turkey, blue arrow for Italy). This difference is accompanied by a lower overall incidence of HCV in the Turkish cohort, resulting in a systematic vertical offset of the two trends in Figure 4b.

Processing the relationship-networked patient clinical data by NPS resulted in novel, more clinically informative and disease-progression based insight into this conventional characterization of overall hepatitis incidence by just the differences in two means. Figure 4 shows, that within the respective HCC progression-ordered phenotype patient subgroups, the incidence of hepatitis exhibits clear trends in both cohorts. HBV incidence in HCC patients is maximal at the early HCC stage patients and decreases proportionally with the HCC progression, defined by the corresponding $CB_s(\tau_s)$ (arrows in Fig. 4a). In contrast, the HCV incidence increases moderately in both cohorts as the HCC progresses to late stages (arrows in Fig. 4b).

The trends of progression of tumor characteristics (mean MTD and number of tumor nodules as the function of corresponding $CB_s(\tau_s)$) are increased in both populations with increasing disease stage (Fig. 5a, Fig. 5b), as expected. They

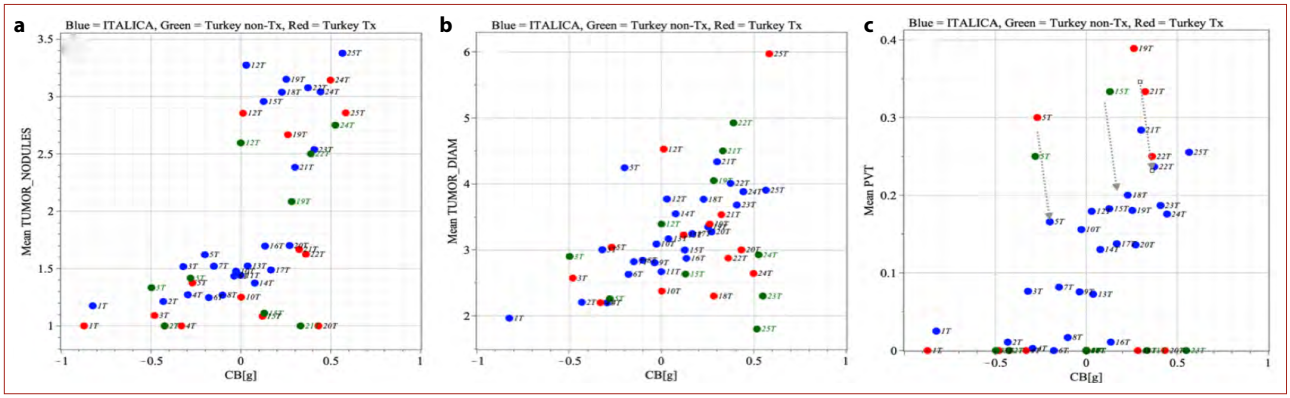

Figure 5. Dependence of phenotype sT-characteristic values of tumor nodule numbers **(a)**, maximal tumor diameters **(b)**, and PVT incidences (vertical axes) on the phenotype characteristic τ_s-dependent clinical burden $CB_s(\tau_s)$ (horizontal axes). Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

are on average parallel in both populations, just with expected moderately smaller tumors in the late HCC stages in Turkish patients, who were selected for liver transplant (Fig. 5b).

In the previous (Italy-based) report (2), we identified 5 groups of 25 HCC progression phenotypes sT, each group with a unique, constant characteristic value of PVT incidence. We found in the Turkish patients (Fig. 5c) a qualitatively similar, PVT-incidence characteristic partitioning of those sT phenotypes, just with quantitatively higher PVT incidence in the top 2 PVT groups in the Turkish than for the Italian patients in the equivalent phenotypes (arrows in Fig. 5c).

Figure 6 shows comparisons of HCC progression for the remaining parallel trends in both groups. Phenotype characteristic total bilirubin and INR values increased with HCC progression (Fig. 6a, 6b), consistent with increasing hepatocyte damage. As the clinical burden increased, the albumin decreased, also consistent with parenchymal destruction by growing tumor. The decreases in both albumin (Fig. 6d) and hemoglobin (Fig. 6e) are consistent with the reported nutritional deficiency and tumor-associated inflammation for growing tumors, as in the Glasgow index.[6] The AFP trended up with increased disease burden in both the Turkish and Italian cohorts (Fig. 6c), but with a large scatter, and with higher absolute values in the Italian than the Turkish cohort. AFP and albumin appear to have an inverse trend (Figs. 6c and 6d), as has been previously shown,[7] as they are in the same family of proteins, with one influencing the other.[8] In this case, standard statistical analysis using absolute values and the NPS approach using trends, showed a very similar result and clinical conclusion in terms of trends. Platelets trended down (Fig. 6f) with increase in tumor burden, likely associated with increasing cirrhosis. For AFP, hemoglobin and albumin, the phenotype characteristic values decrease with HCC progression. The main differences between the Turkish and Italian patients are in relative quantitative shifts of these synchronous trends.

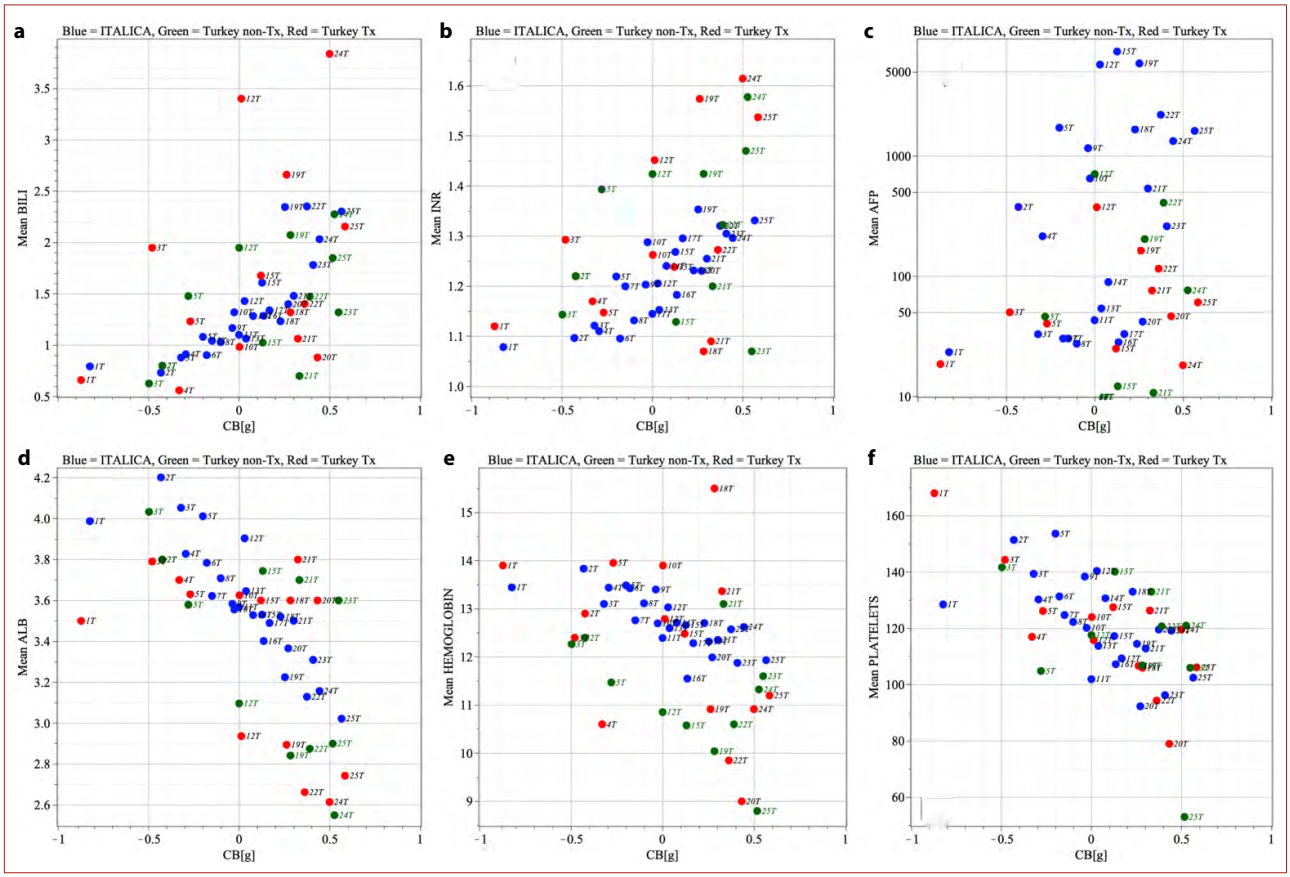

Figure 6. Dependence of phenotype sT-characteristic values of bilirubin **(a)**, INR **(b)**, AFP **(c)**, albumin **(d)**, hemoglobin **(e)** and platelets **(f)** (vertical axes) on the phenotype characteristic τ_s-dependent clinical burden CB_s (τ_s) (horizontal axes). Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

The ALT, AST, ALKP and GGT levels (Fig. 7a-d) are all also systematically higher in the Turkish patients. In addition, in the Turkish patients the phenotype-characteristic ALT, AST and GGT level dependence on HCC progression is opposite (clearly decreasing trend) compared to the Italian patients (moderately increasing trend), and quite similar to the HBV trends of Fig 4. The CB_s (τ_s)-dependent trends of these variables in both cohorts converge to similar characteristic values at CB_s (τ_s)=1. Note also, that in the Italian cohort, there are patients diagnosed in the sT phenotypes, dominantly populated in Turkish patients (1T, 5T, 12T etc.), with the phenotype characteristic levels of these variables and CB_s (τ_s)-dependent trends align ing with the Turkish patients.

A comparison of the outcome characteristics between the Turkish nT and T patients was then made (Fig. 8). Transplant clearly improved survival duration, as expected. New information by NPS is provided by the possibility for studying the trends in survival prolongation as a function of the characteristic HCC progression stage time to

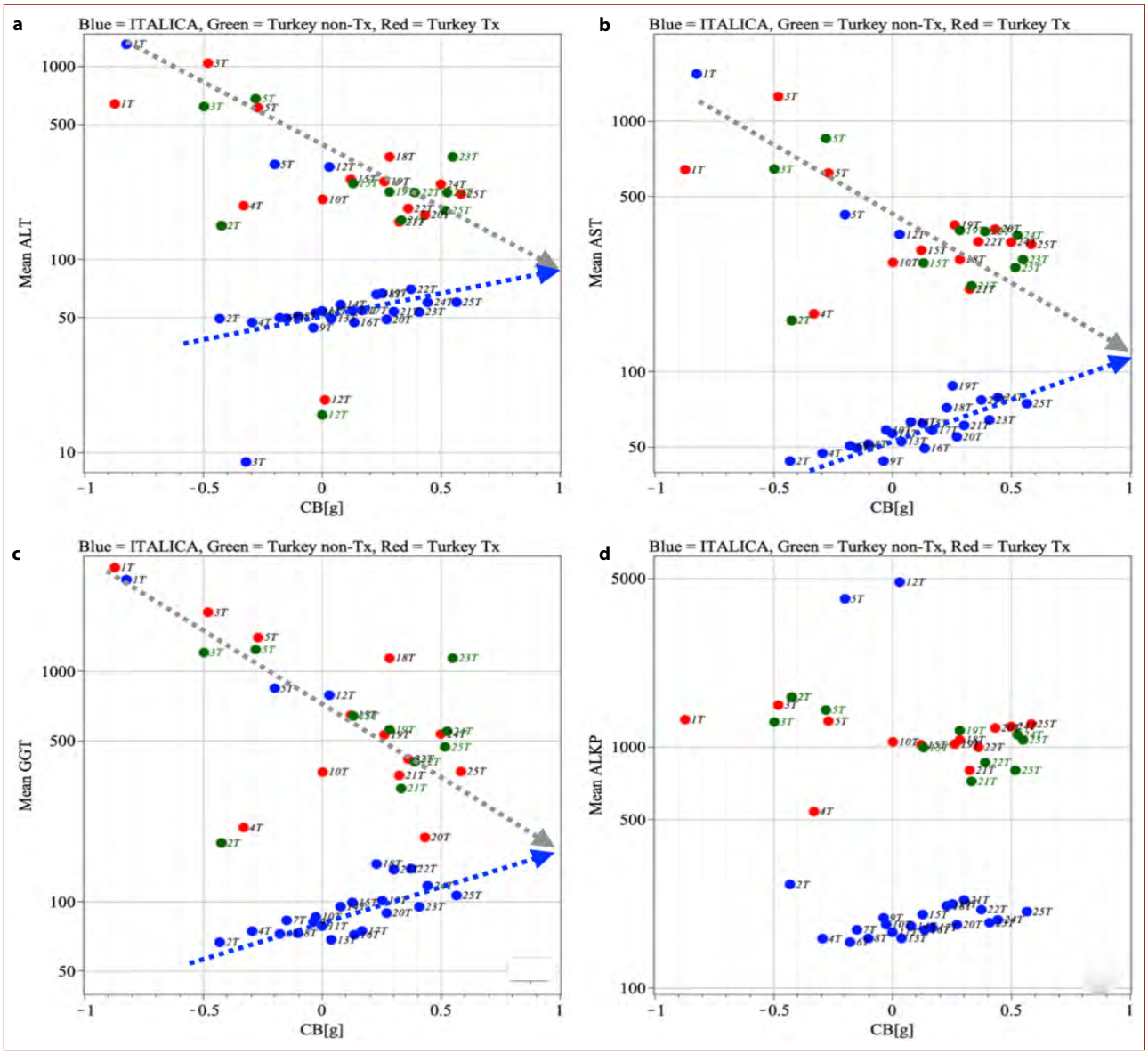

Figure 7. Dependence of phenotype sT-characteristic values of ALT **(a)**, AST **(b)**, GGT **(c)**, and ALKP **(d)** (vertical axes) on the phenotype characteristic τ_s-dependent clinical burden CB_s (τ_s) (horizontal axes). Blue points: data for Italian patients, green points: data for Turkish non-transplant patients, red points: data for Turkish transplant patients.

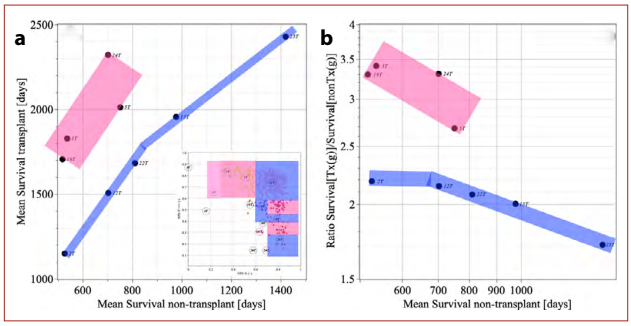

Figure 8. (a) Relationship between the mean survivals of patients in the corresponding HCC phenotypes sT for non-transplant (values on horizontal axis) and transplant (values on vertical axis) groups. Magenta and blue boxes outline the two phenotype subgroups with different survival improvements. The survival improvements in respective groups are also shown by the corresponding color in the HCC progression map (insert). **(b)** Ratios of mean survivals for transplant to mean survivals for no transplant patients in respective sT phenotypes for respective mean survivals in no transplant group. The colors of the boxes are for the same sT groups as in **(a)**.

disease onset τ_s. This shows that there are 2 treatment groups, shown by magenta and blue boxes. In the magenta box, there are larger differences in survival between T and nT patients (e.g. for 5T patients in the magenta box, patients treated with transplant had a mean 2000 day survival, but without transplant they had an approximately 760 day survival, an approximately 2.7 fold survival difference, as shown for 5T in Figure 8b, magenta box. For the 12T phenotype (middle of the trend in the blue box, which is the most populated phenotype, T patients had a 1500 day mean survival, whereas nT patients in the same 12T phenotype had only a 700 day mean survival, or a 2.1 fold survival difference, as shown in 12T in the blue box of Figure 8b. Qualitatively, there are 2 sub-populations with different prolongation ratios (Fig. 8b). In one sub-population, the T patients in the "magenta" progression phenotypes (identified by magenta color in Fig. 8a insert) had a 2.7 to 3.5 times longer typical length of survival than clinically identical nT patients. In the second sub-population in contrast, the T patients in the "blue" progression phenotypes (Fig. 8a insert) had only a 1.7 to 2.2 longer typical length of survival than the clinically identical nT patients. Thus, NPS approach shows the relative treatment advantages for patients, newly diagnosable in multiple phenotypes of HCC that are not differentiable in current clinical practice. The NPS phenotypes treated by T or nT have clinically relevantly different survivals, depending on whether they are in the blue or magenta groups of HCC phenotypes (HCC progression map, see Fig. 8a, insert). In addition, there are NPS-discovered trends in the relative outcomes in both magenta and blue identified phenotype groups, whose patients will benefit more in survival after T compared to nT, depending on phenotype, into which their HCC disease progressed at baseline (Fig. 8).

Logistic Regression Model for Transplant Versus Non-Transplant

Having used NPS to identify the 12T phenotype as containing the majority of the Turkish transplant and non-transplant patients, we then performed standard statistical analysis to identify 6 significant parameters between the 2 Turkish treatment (T and nT) sub-cohorts (Table 1). Table 1 shows the best-performing logical regression model for 12T patients. This recommendation follows from our previous observation (2) that developing the conventional prognostic models for the patients, assigned to clinically identical disease stages by NPS improved the quality, performance and clinical relevance of these models. This improvement follows from removal of randomness in the disease stages in the training "real world" clinical data, which any conventional approach suffers from.

$$P = \frac{1}{(1+e^{-(-4.150-0.724\times gender+0.667\times INR+0.565\times ALB-0.518\times Hb-0.468\times BILI-0.113\times MTD)})}$$

We show above the explicit form of the optimal logistic regression equation for calculating the prognostic value P for transplant treatment eligibility for the most populated Turkish phenotype 12T, using the baseline visit values and weights of 6 best predicting variables, shown in Table 1: If the prognostic value, calculated by substituting the 6 respective personal baseline variable values into the above equation (male code = 0, female code = 1) is P≥0.55, then the patient with diagnosed 12T stage of HCC is categorized as likely to be found eligible for transplant in the Inonu liver transplant institute.

Table 1. Logistic linear regression model for assessing the probability to be selected for liver transplant (threshold = 0.55).

	Intercept	Gender (Female=1)	INR	Alb	Hb	Bilirubin	MTD
Coefficient	-4.150	0.724	-0.667	-0.565	0.518	0.468	0.113
Odds ratio	0.016	2.1	$(2.0)^{-1}$	$(1.8)^{-1}$	1.7	1.6	1.1
p	<0.001	0.006	0.009	0.003	<0.001	<0.001	0.003
Error	1.15	0.4	0.4	0.2	0.06	0.1	0.04

Discussion

NPS provides new information from otherwise "standard" data by adding the conventionally neglected inter-relationship information between the co-observed personal values of 17 clinical variables to the normally used information about only the personal values of respective clinical variables. This leads to clinical analytics approaches standardly using only the population-characterizing mean values (say mean HBV incidence in Italian vs. Turkish cohorts and similarly for bilirubin and other parameters). In contrast, and newly-described here, NPS deconstructs these single value pairs into multiple mean values in respective HCC progression phenotype, sT's, which importantly and again newly, are objectively ordered according to increasing τ_p. Thus, for clinical interpretation, we can improve the conventional frequentist's statistics by discussing actual trends of characteristic clinical variable values as a function of the objectively determined, τ_p-dependent HCC progression stage (Figs. 4-8). These trends are a generalization of the conventional, value-based clinical comparisons (meaning that one can see those conventional overall averages distributed in time into the trends), and are generated by NPS without longitudinal data. This approach has the potential for using the new information in the treatment/no treatment context.

The NPS method is clinically unique because it analytically determines the personal times to disease onset τ_p from each patient's individual baseline data. By selecting the 17 standardized clinical variables as input into the NPS analysis, we also can expect minimal experimental bias in patient characterization in different regions. Conceptually, an important novelty of the NPS approach is in converting the stage characterization, conventionally performed by using the specific values of expert-selected variables, into objective determination of the same physical quantity for any patient with an HCC diagnosis. Consequently, NPS delineated the same phenotypes in the Turkish HCC patients (as it will for any patient cohort with an HCC diagnosis), just as it did in Italian HCC patients. Thus, the quantitative ordering of the NPS diagnosed HCC stages according to their characteristic τ_s permits the study of the τ_s- dependent trends of characteristic clinical variable values without the need for longitudinal data. In this sense, the limited possibilities of future biology of an individual patient's tumor is already encoded in the clinical data from the baseline visit.

We report here (Fig. 9b) that the Turkish patients were diagnosed only in a smaller subset of 16 out of the 25 HCC phenotypes that were populated by the Italian patients (Fig. 9a). These 16 phenotypes, populated exclusively by 100% of the patients in the Turkish cohort are also populated by Italian patients, but these represent only 20-30% of the patient distribution in the Italian cohort.

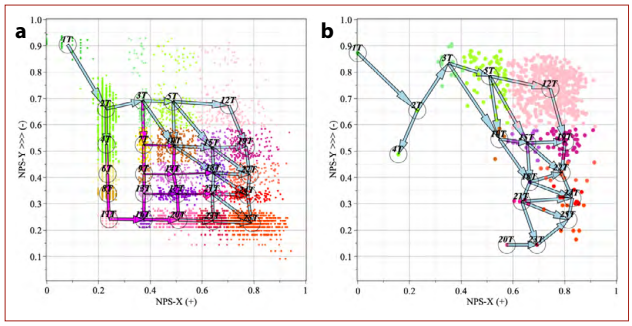

Figure 9. (a) Time-dependent progression between the 25 diagnosed stages for both Italian **(a)** and Turkish **(b)** cohorts. **(a)**, Arrows indicate directions and possible HCC progression paths through NPS identified stage phenotypes sT for Italian patients. Magenta arrows show paths exclusive to the Italian cohort, while gray arrows indicate shared paths for both Italian and Turkish patients. **(b)**, Arrows indicate directions and paths of HCC progression through NPS identified storage phenotypes sT, exclusive to Turkish patients.

Figure 9 shows all possible time-dependent progressions between the 25 diagnosed stages for both the Turkish and Italian cohorts, with the arrows pointing from earlier to the later NPS phenotype in a time-ordered progression. In Figure 9a we show by magenta arrows the model of HCC progression through thew phenotypes, which are exclusive to the Italian population, which we reported previously (2). We have shown that the clinical interpretation of HCC progression of majority of Italian patients (along the magenta arrows) is a dominantly tumor biology-driven process (quantified by the magenta arrows parallel to NPS-Y axis), occurring within a constant extent of liver microenvironmental damage (quantified by the down-oriented magenta arrows parallel to NPS-X axis). In contrast, in the phenotypes shared by the minority of Italian patients and the majority of Turkish patients, the HCC progresses by a more complex disease burden generating mechanism, consisting of simultaneous tumor biology-driven damage, accompanied by advancing liver microenvironmental impairment, i.e. the disease progresses by a more complex mechanism, consisting of simultaneous tumor biology-driven and also by liver microenvironmental (inflammatory) processes. This is represented by the progress along the "diagonal" (light gray) arrows, whose angle and length quantify the extent of simultaneous progression of both factors.

We consider possible reasons for these different findings in the 2 cohorts. The Italian patients were diagnosed predominantly through surveillance of those patients who were known to have chronic hepatitis and were thus considered at risk for HCC development. The Turkish patients had much lower surveillance and as a probable consequence had more advanced HCC stage at diagnosis and thus a

poorer survival in their non-transplant patients. Another reason may be the institutional and treatment-based preselection of patients. This may be related to clinical practice in the healthcare facility, to demographics and to etiology, as there was more HCV in the Italian cohort and more HBV in the Turkish cohort. The Turkish patients also had a greater incidence of males and were typically of younger age.

NPS phenotypes are time-ordered by HCC progression stages, and consequently by the increasing burden of the disease. While Italian patients covered the whole progression interval by being discovered both early, in the middle and also in the final stages of HCC progression dynamics, Turkish patients were represented by the patient sub-group which comes to clinic predominantly in the middle of the progression pathway of the HCC, since they are mostly in phenotype 12T, which is just in the middle of the biological τ_s interval for HCC. We know from the full Italian HCC cohort characterization (2), that 12T is a phenotype in which the patient data shows there to be a simultaneous and quantitatively balanced presence of networked biomarkers of early as well as late HCC stages. In other words, patients in 12T have complex biology and are just in the progression state of HCC when large disease burden biology starts to contribute equally as the early stages with a lesser disease burden biology. So, it is just the time in the disease history, when a clinician might recognize that there is a problem. The ethnic and comorbidities context in the Turkish cohort is what leads to mixed HCC biologies and which also excludes the "simpler" phenotypes, seen in the Italian (but not in the Turkish cohort), since in the Italian cohort only one biology (either early decrease in early stages or late increase in later stages) determined the HCC progression. By contrast, the Turkish patients have both stages changing simultaneously (see Fig. 9b).

Our NPS strategy also led us to observe that in the majority-HBV etiology of the Turkish patients, HBV incidence in HCC patients was maximal at the early HCC stage patients and decreased proportionally with HCC progression (Fig. 4). We consider that this might be explained, either by the HCC growth, initiated within the liver environment already affected by HBV, which in the Turkish cohort is diagnosed practically for all patients in these sT stages, is more extensively destroying parenchyma and thus patients die before their HCCs can get too large, that is a liver death.[9] Alternatively, we consider that the HCCs start to grow only when the HBV disease becomes more advanced (hence the years-long latency between HBV infection and HCC diagnosis), so that these early cancer stage patients die from a hepatitis/liver failure death before the HCC can grow too large. Patients with hepatitis B surface antigen are typically under close follow up and so are more likely to be diagnosed with smaller size tumors, although the literature is mixed on this point.[10-12] This increasing frequency of deaths, caused by the progressing impact of death risk factors in early sT stages, leads to a depletion of the HBV-positive patients from later (t>s) T stages. This results in dominance of cancer biology in defining the clinical profiles and outcomes of patients, which were diagnosed by NPS in those later HCC stages.

Our approach also allowed us to identify 2 treatment-related groups (Fig. 8), and make 2 observations. Firstly, that in four T phenotypes 3T, 5T, 19T and 24T, there was a 2.7 to 3.5 times longer typical length of survival for the transplanted (T) than the non-transplanted (nT) patients who had clinically identical phenotypes (red in Fig. 8a). By contrast, for a separate set of five T phenotypes (2T, 12T, 15T, 22T and 25T), patients (Fig. 8a insert) had only 1.7 to 2.2 longer typical length of survival than clinically identical nT phenotypes (blue in Fig. 8a). Furthermore, in a second observation, we found that some phenotypes benefit more in survival after transplant, even though they all fulfilled the current inclusion criteria for liver transplantation (compare survival for 24T versus 19T patients in the red trend of Fig. 8a). These can only be found after the identification of the 25 phenotypes discovered by the NPS strategy, that cannot otherwise be identified by current standard clinical approaches. As observed elsewhere, only a minority of newly presenting HCC patients are eligible for curative therapies under current guidelines,[13, 14] although the survival differences between surgical and non-surgical treatments for small HCCs can be minor.[15, 16]

Our working hypothesis to explain these results is that for all Turkish Inonu patients, their primary disease is the liver damage, which leads to the (secondary) appearance of HCC. In addition, the damaged liver micro- and macro-environment of HCC tumors in Turkish patients strongly influences the topologies of the relationship networks between the coherently observed values of the 17 variables. This leads to significantly worse survival in the Turkish patients, compared to the surveillance-collected Italian cases.

Conclusion

We show that HCC in Turkish and Italian patients is likely driven by differing processes, the former more complex than the later, even though the Turkish cohort (mainly 12T phenotype) does not catch the whole spectrum of phenotypes (1T to 25T) as found by the nation-wide surveillance strategy in Italy. The fact that only a subset of all HCC phenotypes appears in the Turkish cohort (12T) may be explained by the non-screened HCC patients coming with HCC as a secondary problem, and is primarily driven by the liver environment, which behaves differently to the Italian situation, where HCC is (predominantly) in healthier livers (only ~ 30 % of Italian patients might have the similar environment). Specifically, the Turkish HCC patients are not diagnosed with pheno-

types *6T, 7T, 8T, 9T, 11T, 13T, 14T, 16T, 17T*, which, in contrast are heavily populated by Italian patients.

Note 1. *We made our NPS staging of HCC available through the web tool residing at https://apkatos.github.io/webpage_nps. Accessing the URL provides extension to user local web-browser, so all data handling and processing is done locally at user's computer, without collecting any information or data.*

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.

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

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Authorship Contributions: P.P., Sata Analytics and Writing; B.I.C, Data Collection, Idea and Writing; V.I., Data Collection; S.Y., Paper Review.

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Peer-review: Externally peer-reviewed.

References

1. Pančoška P, Skála L, Nešetřil J, Carr BI. Evaluation of total hepatocellular cancer lifespan, including both clinically evident and preclinical development, using combined network phenotyping strategy and Fisher information analysis. Semin Oncol. 2015 Apr;42(2):339-46. doi: 10.1053/j.seminoncol.2014.12.025. Epub 2015 Jan 5. PMID: 25843738; PMCID: PMC4388062.
2. Carr B, Sotakova B, Pancoska P. A new approach to analysis of clinical data and prognostication for patients with hepatocellular carcinoma, based upon a Network Phenotyping Strategy (NPS) computational method. J. Inonu Liver Transplant Inst. 2024; 2:109-116. Doi: 10.14744/jilti.2024.63935.
3. Wu, J.Q., Horeweg, N., de Bruyn, M. et al. Automated causal inference in application to randomized controlled clinical trials. Nat Mach Intell 4, 436–444 (2022).
4. Frieden BR, Gatenby RA. Principle of maximum Fisher information from Hardy's axioms applied to statistical systems. Phys Rev E Stat Nonlin Soft Matter Phys 2013;88(4):042144.
5. JASP Team (2025). JASP (Version 0.19.3)[Computer software], https://jasp-stats.org/
6. Kinoshita A, Onoda H, Imai N, Iwaku A, Oishi M, Fushiya N, Koike K, Nishino H, Tajiri H. Comparison of the prognostic value of inflammation-based prognostic scores in patients with hepatocellular carcinoma. Br J Cancer. 2012 Sep 4;107(6):988-93. doi: 10.1038/bjc.2012.354. Epub 2012 Aug 9. PMID: 22878374; PMCID: PMC3464773.
7. Carr B, Guerra V, Ince V, Isik B, Yilmaz S. Alpha-fetoprotein and albumin inversely relate to each other and to tumor parameters in patients with hepatocellular carcinoma. Hepatol Forum. 2024 Jan 16;5(1):11-17. doi: 10.14744/hf.2023.2023.0023. PMID: 38283277; PMCID: PMC10809334.
8. Nakata K, Motomura M, Nakabayashi H, Ido A, Tamaoki T. A possible mechanism of inverse developmental regulation of alpha-fetoprotein and albumin genes. Studies with epidermal growth factor and phorbol ester. J Biol Chem. 1992;267(2):1331-4. PMID: 1370467.
9. Couto OF, Dvorchik I, Carr BI. Causes of death in patients with unresectable hepatocellular carcinoma. Dig Dis Sci. 2007 Nov;52(11):3285-9. doi: 10.1007/s10620-007-9750-3. Epub 2007 Apr 10. PMID: 17436087.
10. Roayaie S, Haim MB, Emre S, Fishbein TM, Sheiner PA, Miller CM, Schwartz ME. Comparison of surgical outcomes for hepatocellular carcinoma in patients with hepatitis B versus hepatitis C: a western experience. Ann Surg Oncol. 2000 Dec;7(10):764-70. doi: 10.1007/s10434-000-0764-8. PMID: 11129425.
11. Aljumah AA, Kuriry H, Faisal N, Alghamdi H. Clinicopathologic characteristics andoutcomes of hepatocellular carcinoma associated with chronic hepatitis B versus hepatitis C infection. Ann Saudi Med. 2018 Sep-Oct;38(5):358-365. doi: 10.5144/0256-4947.2018.358. PMID: 30284991; PMCID: PMC6180214.
12. Franssen B, Alshebeeb K, Tabrizian P, Marti J, Pierobon ES, Lubezky N, Roayaie S, Florman S, Schwartz ME. Differences in surgical outcomes between hepatitis B- and hepatitis C-related hepatocellular carcinoma: a retrospective analysis of a single North American center. Ann Surg. 2014 Oct;260(4):650-6; discussion 656-8. doi: 10.1097/SLA.0000000000000917. PMID: 25203882.
13. Chen X, Liu HP, Li M, Qiao L. Advances in non-surgical management of primary liver cancer. World J Gastroenterol. 2014 Nov 28;20(44):16630-8. doi: 10.3748/wjg.v20.i44.16630. PMID: 25469032; PMCID: PMC4248207.
14. Zhang X, El-Serag HB, Thrift AP. Predictors of five-year survival among patients with hepatocellular carcinoma in the United States: an analysis of SEER-Medicare. Cancer Causes Control. 2021 Apr;32(4):317-325. doi: 10.1007/s10552-020-01386-x. Epub 2021 Jan 4. PMID: 33394207.
15. Nomura A, Ishigami M, Honda T, Kuzuya T, Ishizu Y, Ito T, Kamei H, Onishi Y, Ogura Y, Fujishiro M. Limitation of non-transplant treatment and proper timing for liver transplantation in patients with hepatocellular carcinoma considering long-term survival. Medicine (Baltimore). 2020 Jul 10;99(28):e21161.
16. Midorikawa Y, Takayama T, Shimada K, Nakayama H, Higaki T, Moriguchi M, Nara S, Tsuji S, Tanaka M. Marginal survival benefit in the treatment of early hepatocellular carcinoma. J Hepatol. 2013 Feb;58(2):306-11. doi: 10.1016/j.jhep.2012.09.026. Epub 2012 Oct 9. PMID: 23063418.

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Original Research

Unraveling Transcriptomic Differences in Hepatocellular Carcinoma and Intrahepatic Cholangiocarcinoma Through RNA-Seq and Functional Enrichment

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Abstract

Objectives: Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) are the most common primary liver cancers that differ in origin and histopathological features. This study aimed to identify the differentially expressed genes and dysregulated pathways between HCC and iCCA using transcriptomic and bioinformatics analyses.

Methods: Gene expression data were obtained from the GEO database (accession number GSE241466), comprising RNA-seq profiles from 3 HCC and 5 iCCA tumor samples. Data were processed and analyzed using the *limma* package in R, applying a |log2 fold change| > 1 and adjusted p-value < 0.05 as significance thresholds. Visualization techniques including UMAP, volcano plots, were employed. Functional enrichment analysis of DEGs was conducted via the *clusterProfiler* package, integrating gene ontology terms and gene-concept network plots to explore relevant biological processes and molecular functions.

Results: Out of 17,637 genes, a total of 1,248 genes were found to be significantly differentially expressed between HCC and iCCA. UMAP analysis demonstrated clear clustering and separation between the two cancer types. Enrichment analyses revealed key biological differences, notably in metabolic reprogramming, extracellular matrix organization, and neuron projection development. Notably, genes such as *NLGN1*, *EPHA6*, and *SEMA3E*, involved in neural differentiation and signaling, were significantly enriched in iCCA, suggesting a potential role of neuron-like features in its progression. Conversely, HCC samples were characterized by upregulation of genes linked to amino acid metabolism and hepatocellular-specific functions.

Conclusion: This study elucidates the molecular divergence between HCC and iCCA, identifying distinct gene expression profiles and enriched biological pathways. The activation of neural signaling pathways in iCCA, coupled with differential engagement of metabolic and morphogenetic processes, suggests subtype-specific mechanisms that could inform future diagnostic and therapeutic strategies. These findings provide a foundation for the development of tailored clinical interventions in primary liver cancers.

Keywords: Differential gene expression, gene ontology, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, RNA-seq, tumor biology

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Primary liver cancer ranks among the most common malignancies globally and is recognized for its aggressive behavior and high mortality rate. Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (iCCA) together account for approximately 75- 85% and 15% of all primary liver cancers, respectively.[1-4] In recent years, the incidence of both HCC and iCCA has shown a marked increase, particularly in Western countries. Although both originate within the liver, they differ substantially in their morphological characteristics, patterns of metastasis, and therapeutic responsiveness.[5, 6]

HCC typically exhibits a solid, trabecular, or occasionally pseudoglandular growth pattern, with dense tumor cell populations and minimal desmoplastic stroma. By contrast, iCCA is characterized by ductular, papillary, or solid architectural patterns embedded within a prominent fibrotic tumor stroma.[7] While HCC is inherently invasive, iCCA demonstrates a higher propensity for the development of distant metastases. Notably, HCCs often respond favorably to treatment with multikinase inhibitors, whereas iCCAs tend to be resistant to these targeted therapies. Conversely, iCCAs are more sensitive to conventional cytotoxic chemotherapy, whereas HCCs generally display chemotherapy resistance.[8, 9]

Although iCCA is less common compared to HCC, its incidence is rising globally, especially in regions with a high prevalence of chronic liver conditions, including hepatitis C infection and cirrhosis.[10, 11] iCCA is associated with a particularly poor prognosis, with reported five-year survival rates following curative resection ranging between 22–24%.[12] The increasing occurrence of iCCA is attributed to a combination of risk factors such as nonalcoholic fatty liver disease, chronic viral hepatitis, and primary sclerosing cholangitis.[13-16]

Clinically, iCCA often presents with nonspecific symptoms, and many cases are incidentally detected during imaging performed for unrelated reasons. When present, symptoms may include unintentional weight loss, jaundice, abdominal pain, and fever. However, the vague nature of these symptoms frequently leads to delays in diagnosis.[17, 18] Imaging studies such as computed tomography (CT) and magnetic resonance imaging (MRI) typically reveal a mass with homogeneous low attenuation and irregular peripheral enhancement, occasionally accompanied by capsular retraction and intrahepatic ductal dilatation.[19, 20] Definitive diagnosis usually requires histopathological confirmation through biopsy or surgical specimen analysis.

Molecularly, HCC and iCCA are driven by distinct oncogenic pathways and genetic alterations. HCC is frequently associated with mutations in genes such as TP53, CTNNB1 (β-catenin), and AXIN1, which are involved in cell cycle regulation, Wnt/β-catenin signaling, and genomic stability.[21] In contrast, iCCA often harbors alterations in genes like IDH1/2, FGFR2, KRAS, and ARID1A, reflecting its origin from biliary epithelial cells and its reliance on different tumorigenic mechanisms.[22] These genetic discrepancies not only contribute to the diverse histopathological features of HCC and iCCA but also underpin their variable responses to systemic therapies and prognostic outcomes. Understanding these fundamental molecular distinctions is critical for developing subtype-specific diagnostic, prognostic, and therapeutic strategies.

Given the distinct biological behaviors, morphological features, and clinical outcomes of HCC and iCCA, elucidating their underlying molecular differences has become increasingly important. In this context, our study aimed to perform a comprehensive transcriptomic analysis using publicly available data, applying bioinformatics tools such as differential gene expression analysis and gene ontology enrichment analysis. Through these approaches, we sought to identify key genes and biological pathways that differentiate HCC from iCCA, thereby contributing to a deeper understanding of the molecular mechanisms driving these two primary liver cancer subtypes and highlighting potential targets for future diagnostic and therapeutic strategies.

Methods

Dataset

The transcriptomic profiling data utilized in this study were obtained from the NCBI Gene Expression Omnibus (GEO) database under the accession number GSE241466. This publicly available dataset includes gene expression profiles derived from tumor tissue samples collected from patients diagnosed with HCC and iCCA. Specifically, the dataset comprises samples from three HCC patients and five iCCA patients. RNA sequencing was performed to capture the comprehensive transcriptomic landscape of these primary liver cancers. The high-throughput sequencing data were processed and normalized to enable downstream differential expression and functional enrichment analyses.

RNA-Sequence Analysis (RNA-Seq)

RNA sequencing (RNA-seq) has become a transformative technology in transcriptomics, offering a detailed and quantitative assessment of all RNA species expressed within a cell or tissue at a specific point in time. As a major advancement over traditional hybridization-based methods, RNA-seq not only quantifies known transcripts with high sensitivity and precision but also enables the discovery of novel transcripts, alternative splice variants, and previously

unidentified RNA molecules. This capacity makes RNA-seq particularly valuable for investigating the complex regulatory networks governing gene expression under various biological contexts.

A key advantage of RNA-seq is its exceptionally wide dynamic range, allowing for the accurate detection of transcripts across a broad spectrum of expression levels. Additionally, RNA-seq is characterized by minimal background noise and high signal specificity, thereby improving the robustness and reproducibility of gene expression measurements. The technology also supports the fine-scale mapping of exon-intron boundaries and facilitates the identification of genetic variations, such as single nucleotide polymorphisms (SNPs) and small insertions or deletions (indels), within expressed sequences. Due to its versatility, precision, and depth of analysis, RNA-seq has become a cornerstone technique in molecular biology research, particularly in areas such as gene discovery, functional genomics, and the study of transcriptomic changes associated with disease processes.[23, 24]

Bioinformatics and Gene Expression Analysis

Bioinformatics is an interdisciplinary science dedicated to the systematic collection, storage, management, analysis, and visualization of biological and medical data. It employs both theoretical frameworks and applied methodologies across fields such as biology, medicine, behavioral sciences, and public health. The primary goal of bioinformatics is to develop and refine computational tools and strategies that facilitate the effective utilization and interpretation of experimental and observational data generated through scientific research or established protocols. Analyses are performed by selecting the most suitable databases and computational technologies based on the biological problem, molecule, or structure of interest. The resulting interpretations are cross-referenced with prior studies to ensure scientific accuracy and contextual relevance.[25, 26]

Alterations in the physiological state of a cell or organism are frequently accompanied by changes in gene expression profiles, making gene expression analysis a cornerstone of contemporary biological research. DNA microarray technology, despite ongoing advancements, remains a pivotal technique for evaluating gene expression. This method relies on the hybridization of mRNA molecules to a dense array of immobilized DNA probes, each corresponding to a specific gene. It enables researchers to investigate how gene expression patterns are modulated by various chemical agents, thereby providing insights into their biological activities and potential toxicities. Furthermore, comparative analysis of clinical samples from healthy individuals and patients facilitates the discovery of novel biomarkers, which are crucial for improving early disease detection, predicting clinical outcomes, and guiding the development of targeted therapeutic strategies.[27, 28]

Bioinformatics Analysis Phase

In this study, bioinformatics analyses were conducted using a publicly available transcriptomic dataset to identify genes and biological pathways potentially distinguishing HCC from iCCA. The analyses were carried out utilizing the limma package—an R-based statistical tool developed for the analysis of gene expression data through linear modeling techniques.[29] Although originally designed for microarray studies, limma has been widely adapted for use with RNA sequencing (RNA-seq) datasets through appropriate normalization methods.

One of the key strengths of the limma approach is its application of Empirical Bayes moderation, which improves statistical power, particularly when analyzing datasets with relatively small sample sizes. In the current analysis, the log2 fold change (log2FC) was calculated to measure the degree of differential expression between HCC and iCCA groups. Genes with a log2FC greater than 1 were considered significantly upregulated, whereas those with a log2FC less than -1 were considered significantly downregulated.

To assess the overall distribution of gene expression values across samples, box plots and density plots were generated. These visualizations confirmed consistency across samples, indicating reliable data quality suitable for downstream analyses. Furthermore, Uniform Manifold Approximation and Projection (UMAP) was employed to visualize sample clustering based on transcriptomic profiles, effectively distinguishing HCC from iCCA groups and demonstrating the intrinsic biological variability between the tumor types.

Differentially expressed genes were visualized through a volcano plot, which allowed simultaneous interpretation of the magnitude (log2FC) and statistical significance (-log10 p-value) of gene expression changes. In this plot, upregulated genes are represented in red, downregulated genes in blue, and non-significant genes in black, facilitating the rapid identification of key molecular differences.

Subsequently, gene ontology enrichment analysis was performed using the clusterProfiler package to identify overrepresented biological processes, molecular functions, and cellular components among the differentially expressed genes. Human gene annotations were sourced from the org.Hs.eg.db database to ensure accurate gene-term mappings.

For visualization of enrichment results, multiple graphical approaches were utilized, including dot plots, enrichment maps (emapplots), gene-concept networks (cnetplots), and gene set enrichment analysis (GSEA) plots, leveraging

the capabilities of the enrichplot and DOSE packages. To enhance aesthetic quality and interpretability, visualizations were further customized using ggplot2.

Gene set enrichment analysis was based on the ranked list of genes according to log2FC values, with a stringent statistical threshold of p<0.05 applied to maintain the robustness and biological relevance of the findings.

Collectively, these bioinformatics analyses revealed key functional categories and biological pathways associated with the distinct gene expression patterns observed between HCC and iCCA, offering new insights into their molecular pathogenesis.

Results

Figure 1 illustrates the UMAP (Uniform Manifold Approximation and Projection) plot, offering a visual representation of the clustering behavior and relationships among the analyzed samples. As a powerful dimensionality reduction method, UMAP enables the identification of patterns and distinctions within complex, high-dimensional gene expression datasets. In this plot, samples with comparable molecular features are observed to cluster closely together, highlighting that the transcriptomic profiles of HCC and iCCA are markedly distinct. Green dots represent HCC samples, while purple dots denote iCCA samples. The clear separation between these clusters suggests significant underlying biological differences between the two tumor types. This visual segregation validates the appropriateness of subsequent differential gene expression analyses. UMAP's ability to preserve both global and local data structures in a low-dimensional space proves invaluable for initial exploratory analyses, providing strong preliminary evidence of transcriptomic divergence and supporting the biological relevance of the dataset for further investigation.

Figure 2 shows the results of the differential gene expression analysis performed using Limma to compare HCC and iCCA samples. An adjusted p-value threshold of Padj <0.05 was applied to ensure the identification of statistically significant gene expression differences. Out of a total of 17,637 genes analyzed, 1,248 genes were found to be differentially expressed between HCC and iCCA. This substantial number of differentially expressed genes reflects distinct molecular signatures characterizing each tumor type. The identification of these genes provides important insights into the divergent biological pathways and mechanisms underlying HCC and iCCA pathogenesis. These results form the basis for subsequent functional enrichment analyses aimed at uncovering key biological processes and potential biomarkers that differentiate these two forms of primary liver cancer.

Figure 3 presents the volcano plot, offering a comprehensive visualization of the genes that are significantly differentially expressed between HCC and iCCA samples. This plot is a standard tool in transcriptomic analyses, enabling simultaneous evaluation of both the magnitude of gene expression changes (log2 fold change) and their statistical significance (p-value). In this representation, each dot cor-

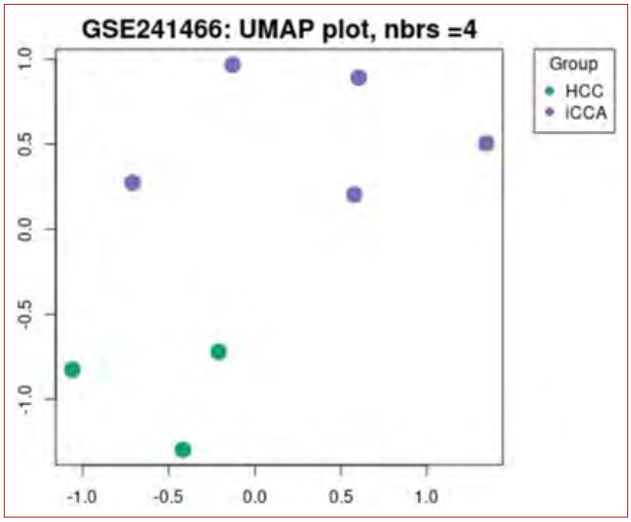

Figure 1. UMAP plot of the samples (Green Dots: HCC tissues, Purple dots: iCCA tissues).

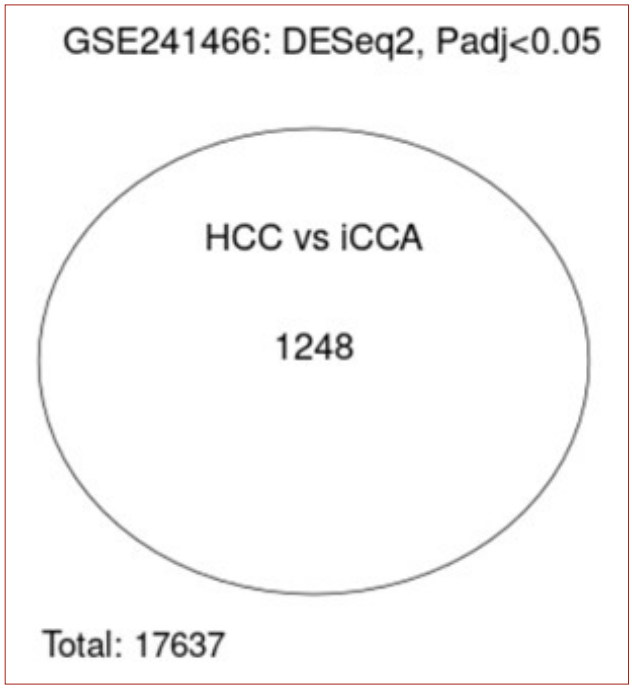

Figure 2. Differentially expressed genes between HCC and iCCA tissues (Padj < 0.05).

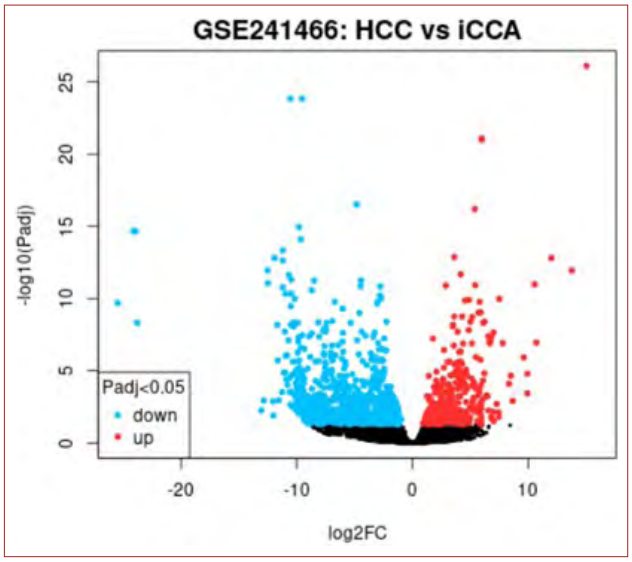

Figure 3. Volcano plot of genes in HCC and iCCA tissues. (Red dots represent transcripts that increased, blue dots represent transcripts that dropped, and black dots represent transcripts whose expression level remained unchanged.)

responds to an individual gene, where the x-axis displays the log2 fold change (log2FC), reflecting the degree of upregulation or downregulation, while the y-axis shows the -log10(p-value), indicating the confidence level of the observed changes. Genes that surpass the defined thresholds (|log2FC| > 1.0 and Padj < 0.05) are visually emphasized, facilitating the distinction of significantly upregulated and downregulated genes between HCC and iCCA. This plot provides critical insights into the molecular differences driving the distinct biological behaviors of these two liver cancer types and highlights candidate genes that may serve as biomarkers or therapeutic targets for future investigations.

Figures 4, 5, and 6 summarize the gene ontology enrichment analysis results for differentially expressed genes identified in HCC and iCCA samples.

In Figure 4, a dot plot illustrates enriched biological processes among activated and suppressed gene sets. The x-axis represents the GeneRatio (the ratio of input genes annotated to a given gene ontology term), while dot size indicates the number of associated genes. The color gradient corresponds to the adjusted p-value (p.adjust), with red shades denoting more significant enrichment. Within the activated gene set, significant biological processes include cellular metabolic pathways (such as organic acid catabolic process, amino acid metabolism) and morphogenetic processes (such as cell part morphogenesis and neuron projection development). In contrast, the suppressed gene set is enriched in processes

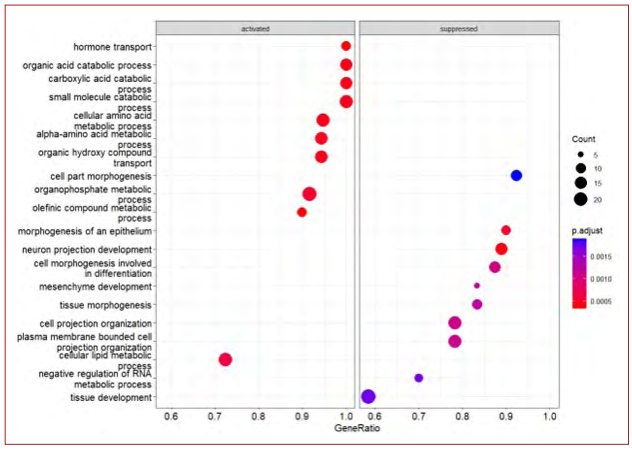

Figure 4. Gene ontology enrichment analysis for HCC and iCCA tissues groups.

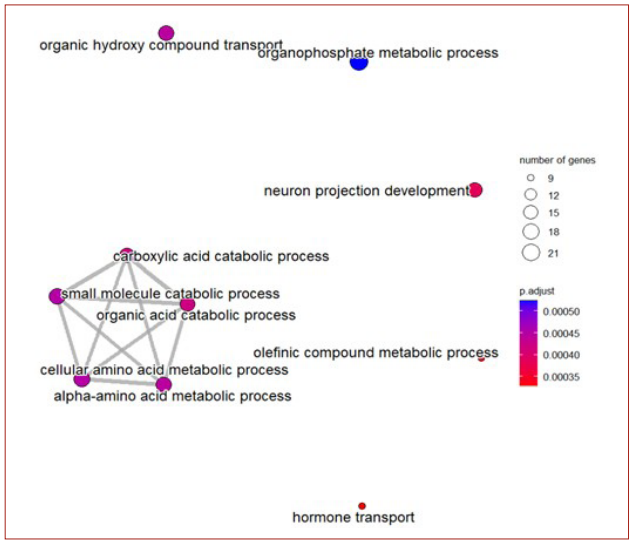

Figure 5. Network map of semantic relationships between gene ontology terms.

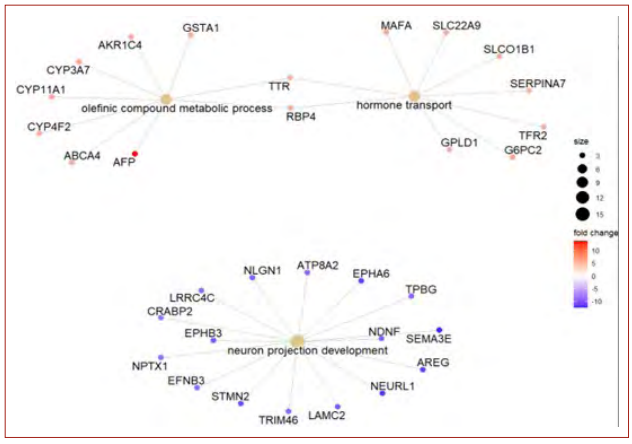

Figure 6. Gene-Process Network.

related to RNA regulation and tissue development, suggesting the differential engagement of growth and differentiation pathways between HCC and iCCA.

Figure 5 provides a network view of the enriched gene ontology terms, revealing the functional interrelations among biological processes. Metabolic processes, particularly related to small molecule and organic acid catabolism, form a densely interconnected cluster. Importantly, "neuron projection development" appears as an isolated yet significantly enriched biological process, suggesting that neuronal-like differentiation pathways may be uniquely activated in certain subgroups, potentially more prominently in iCCA.

Figure 6 extends the analysis by mapping specific differentially expressed genes onto their corresponding biological processes. In particular, genes such as NLGN1, ATP8A2, EPHA6, TPBG, and SEMA3E are associated with neuron projection development, indicating a potential involvement of neural differentiation pathways. Meanwhile, olefinic compound metabolism and hormone transport processes are connected to genes such as AFP, CYP3A7, and RBP4, known to play roles in liver-specific metabolic functions.

Overall, these findings highlight distinct biological pathways that differentiate HCC and iCCA, with a notable activation of neuronal development processes in iCCA-related profiles. The enrichment of neuron projection-related genes supports the hypothesis that iCCA may exploit neural-like cellular mechanisms during tumor progression, potentially contributing to its distinct biological behavior compared to HCC.

Discussion

Primary liver cancers, namely HCC and iCCA, represent biologically and clinically distinct malignancies arising within the liver. Although both entities originate from hepatic tissue, they differ substantially in morphology, metastatic behavior, treatment response, and molecular pathogenesis. HCC typically arises from hepatocytes and is frequently associated with mutations in TP53, CTNNB1, and AXIN1, involving pathways related to cell cycle regulation and Wnt/β-catenin signaling.[30, 31] In contrast, iCCA is believed to originate from biliary epithelial cells and harbors mutations in IDH1/2, FGFR2, KRAS, and ARID1A, reflecting alternative oncogenic mechanisms.[32] These genetic differences underpin the histopathological diversity and therapeutic challenges observed between HCC and iCCA.

In our study, we performed a comprehensive transcriptomic analysis to explore the molecular divergences between HCC and iCCA, employing differential gene expression and gene ontology enrichment analyses to uncover key biological pathways involved in their pathogenesis.

The functional enrichment analysis of differentially expressed genes revealed profound biological differences, particularly involving metabolic reprogramming, morphogenesis, and neuronal-like differentiation.

a. Metabolic Reprogramming and Tumor Adaptation

As illustrated in the enrichment analysis (Fig. 4), genes activated in both HCC and iCCA are significantly enriched in metabolic processes, including organic acid catabolic process, carboxylic acid metabolism, and small molecule catabolism. These findings highlight a shared but critical adaptation mechanism, where tumors rewire their metabolism to thrive under nutrient-deprived and oxidative stress conditions. The prominent enrichment of amino acid metabolism, particularly cellular amino acid metabolic processes, reflects the reliance of these cancers on alternative metabolic substrates to sustain rapid proliferation.

In contrast, suppression of RNA metabolic processes and tissue development pathways suggests a profound dysregulation of liver and biliary homeostasis, emphasizing the dedifferentiated and aggressive nature of these tumors.

b. Neuron Projection Development: A Distinctive Feature in iCCA

A striking finding was the significant enrichment of neuron projection development pathways, predominantly in iCCA-related gene expression profiles (Figs. 4, 5, and 6). Network analysis demonstrated that neuron projection development forms a separate cluster from the major metabolic pathways. Genes such as NLGN1, ATP8A2, EPHA6, TPBG, and SEMA3E—classically associated with axonal growth and neural communication—were highly represented in this pathway.

This suggests that iCCA tumors may adopt neural-like signaling programs to promote invasion, migration, and cellular plasticity. Such neural features are clinically relevant, as perineural invasion is frequently observed in iCCA and is associated with poor prognosis. This neuronal program may thus be a key contributor to the distinct metastatic behavior observed in iCCA compared to HCC.

c. Morphogenesis and Extracellular Remodeling

Beyond metabolic adaptation, both tumor types showed enrichment in pathways related to tissue morphogenesis, cell projection organization, and epithelial morphogenesis (Figs. 4 and 5).

Genes involved in extracellular matrix remodeling and adhesion processes, including LAMC2, TRIM46, and EFNB3, were differentially expressed, suggesting active reorganization of the tumor microenvironment.

This biological remodeling is mirrored histologically: HCC often demonstrates trabecular or pseudoglandular growth patterns with minimal stroma, whereas iCCA typically exhibits glandular structures embedded in dense desmoplastic stroma. The molecular activation of morphogenetic pathways likely underlies these distinct histological patterns.

d. Clinical Implications and Future Directions

These transcriptomic and functional insights underscore the biological divergence between HCC and iCCA. While metabolic rewiring appears as a shared survival strategy, the distinct activation of neuronal projection pathways in iCCA opens promising avenues for future therapeutic strategies targeting neural-like signaling and perineural invasion.

Moreover, the emphasis on extracellular matrix remodeling suggests that anti-fibrotic therapies or treatments targeting stromal components could potentially enhance the efficacy of conventional therapies, particularly in iCCA, where desmoplasia presents a significant barrier to drug delivery.

In conclusion, our integrative analysis highlights the complex interplay between metabolic adaptation, cellular plasticity, and extracellular matrix dynamics in primary liver cancers. Understanding these distinct biological processes provides a framework for the development of subtype-specific diagnostic markers and therapeutic interventions, ultimately contributing to more personalized and effective management strategies for HCC and iCCA.

This study provides a comprehensive comparative analysis of HCC and iCCA at the transcriptomic level, revealing profound molecular divergences that underpin their distinct biological behaviors. Through differential gene expression and functional enrichment analyses, we identified key pathways differentiating the two tumor types, notably in metabolic reprogramming, extracellular matrix organization, and neuron projection development.

Understanding these distinct molecular signatures not only enhances our knowledge of liver cancer biology but also underscores the necessity for subtype-specific diagnostic and therapeutic approaches. Future research focusing on the targeting of metabolic pathways, stromal interactions, and neuron-like invasive mechanisms may offer novel strategies for improving outcomes in patients with primary liver cancers.

Disclosures

Ethics Committee Approval: This article was produced from NCBI open-access dataset. Therefore, it has been reported by the institute that ethics committee approval is not required.

Conflict of Interest: None declared.

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References

1. Marquardt JU, Andersen JB, Thorgeirsson SS. Functional and genetic deconstruction of the cellular origin in liver cancer. Nature reviews Cancer 2015; 15(11): 653-667 [PMID: 26493646 DOI: 10.1038/nrc4017]
2. Adhoute X, Pietri O, Pénaranda G, Wolf T, Beaurain P, Monnet O, Laquière A, Bonomini J, Neumann F, Levrel O, Buono JP, Hanna X, Castellani P, Perrier H, Bourliere M, Anty R. Intrahepatic Cholangiocarcinoma and Hepatocellular Carcinoma: Real-life Data on Liver Disease, Treatment and Prognosis. J Clin Transl Hepatol 2023; 11(5): 1106-1117 [PMID: 37577232 PMCID: PMC10412698 DOI: 10.14218/jcth.2022.00141]
3. Banales JM, Marin JJG, Lamarca A, Rodrigues PM, Khan SA, Roberts LR, Cardinale V, Carpino G, Andersen JB, Braconi C, Calvisi DF, Perugorria MJ, Fabris L, Boulter L, Macias RIR, Gaudio E, Alvaro D, Gradilone SA, Strazzabosco M, Marzioni M, Coulouarn C, Fouassier L, Raggi C, Invernizzi P, Mertens JC, Moncsek A, Ilyas SI, Heimbach J, Koerkamp BG, Bruix J, Forner A, Bridgewater J, Valle JW, Gores GJ. Cholangiocarcinoma 2020: the next horizon in mechanisms and management. Nat Rev Gastroenterol Hepatol 2020; 17(9): 557-588 [PMID: 32606456 PMCID: PMC7447603 DOI: 10.1038/s41575-020-0310-z]
4. Gingold JA, Zhu D, Lee DF, Kaseb A, Chen J. Genomic Profiling and Metabolic Homeostasis in Primary Liver Cancers. Trends Mol Med 2018; 24(4): 395-411 [PMID: 29530485 DOI: 10.1016/j.molmed.2018.02.006]
5. Pascale A, Rosmorduc O, Duclos-Vallée JC. New epidemiologic trends in cholangiocarcinoma. Clinics and research in hepatology and gastroenterology 2023; 47(9): 102223 [PMID: 37797807 DOI: 10.1016/j.clinre.2023.102223]
6. Rimassa L, Khan S, Groot Koerkamp B, Roessler S, Andersen JB, Raggi C, Lleo A, Nault JC, Calderaro J, Gabbi C, Kather JN, Banales JM, Bargellini I, Morement H, Krawczyk M, Farazi PA, Carpino G, Avila MA, Saborowski A, Cardinale V, Braconi C, Macias RIR. Mapping the landscape of biliary tract cancer in Europe: challenges and controversies. The Lancet regional health Europe 2025; 50: 101171 [PMID: 40093398 PMCID: PMC11910794 DOI: 10.1016/j.lanepe.2024.101171]
7. Goodman ZD. Neoplasms of the liver. Modern Pathol 2007; 20(1): S49-S60 [PMID: 17486052 DOI: 10.1038/modpathol.3800682]
8. Marin JJ, Macias RI, Monte MJ, Romero MR, Asensio M, Sanchez-Martin A, Cives-Losada C, Temprano AG, Espinosa-Escudero R, Reviejo M. Molecular bases of drug resistance in hepatocellular carcinoma. Cancers (Basel) 2020; 12(6): 1663 [PMID: 32585893 PMCID: PMC7352164 DOI: 10.3390/cancers12061663]

9. Marin JJ, Lozano E, Herraez E, Asensio M, Di Giacomo S, Romero MR, Briz O, Serrano MA, Efferth T, Macias RI. Chemoresistance and chemosensitization in cholangiocarcinoma. Biochim Biophys Acta Mol Basis Dis 2018; 1864(4): 1444-1453 [PMID: 28600147 DOI: 10.1016/j.bbadis.2017.06.005]
10. Li H, Hu B, Zhou Z-Q, Guan J, Zhang Z-Y, Zhou G-W. Hepatitis C virus infection and the risk of intrahepatic cholangiocarcinoma and extrahepatic cholangiocarcinoma: evidence from a systematic review and meta-analysis of 16 case-control studies. World J Surg Oncol 2015; 13: 1-8 [PMID: 25903488 PMCID: PMC4419416 DOI: 10.1186/s12957-015-0583-9]
11. Massarweh NN, El-Serag HB. Epidemiology of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Cancer Control 2017; 24(3): 1073274817729245 [PMID: 28975830 PMCID: PMC5937247 DOI: 10.1177/1073274817729245]
12. Schwenk L, Rohland O, Deeb AA, Dondorf F, Settmacher U, Rauchfuß F. Liver Transplantation for Incidental Cholangiocarcinoma or Combined Hepatocellular Carcinoma/Cholangiocarcinoma-Own Experiences and Review of the Literature. Cancers (Basel) 2023; 15(14): 3609 [PMID: 37509271 PMCID: PMC10377009 DOI: 10.3390/cancers15143609]
13. De Lorenzo S, Tovoli F, Mazzotta A, Vasuri F, Edeline J, Malvi D, Boudjema K, Renzulli M, Jeddou H, D'Errico A, Turlin B, Cescon M, Uguen T, Granito A, Lièvre A, Brandi G. Non-Alcoholic Steatohepatitis as a Risk Factor for Intrahepatic Cholangiocarcinoma and Its Prognostic Role. Cancers (Basel) 2020; 12(11) [PMID: 33138044 PMCID: PMC7692633 DOI: 10.3390/cancers12113182]
14. Wongjarupong N, Assavapongpaiboon B, Susantitaphong P, Cheungpasitporn W, Treeprasertsuk S, Rerknimitr R, Chaiteerakij R. Non-alcoholic fatty liver disease as a risk factor for cholangiocarcinoma: a systematic review and meta-analysis. BMC gastroenterology 2017; 17(1): 149 [PMID: 29216833 PMCID: PMC5721586 DOI: 10.1186/s12876-017-0696-4]
15. Clements O, Eliahoo J, Kim JU, Taylor-Robinson SD, Khan SA. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: A systematic review and meta-analysis. J Hepatol 2020; 72(1): 95-103 [PMID: 31536748 DOI: 10.1016/j.jhep.2019.09.007]
16. Tyson GL, El-Serag HB. Risk factors for cholangiocarcinoma. Hepatology (Baltimore, Md) 2011; 54(1): 173-184 [PMID: 21488076 PMCID: PMC3125451 DOI: 10.1002/hep.24351]
17. Kodali S, Connor AA, Brombosz EW, Ghobrial RM. Update on the Screening, Diagnosis, and Management of Cholangiocarcinoma. Gastroenterology & hepatology 2024; 20(3): 151-158 [PMID: 38680168 PMCID: PMC11047158]
18. Brown KM, Parmar AD, Geller DA. Intrahepatic cholangiocarcinoma. Surgical oncology clinics of North America 2014; 23(2): 231-246 [PMID: 24560108 PMCID: PMC4007210 DOI: 10.1016/j.soc.2013.10.004]
19. Chung YE, Kim M-J, Park YN, Choi J-Y, Pyo JY, Kim YC, Cho HJ, Kim KA, Choi SY. Varying appearances of cholangiocarcinoma: radiologic-pathologic correlation. Radiographics 2009; 29(3): 683-700 [PMID: 19448110 DOI: 10.1148/rg.293085729]
20. Kovač JD, Janković A, Đikić-Rom A, Grubor N, Antić A, Dugalić V. Imaging Spectrum of Intrahepatic Mass-Forming Cholangiocarcinoma and Its Mimickers: How to Differentiate Them Using MRI. Current oncology (Toronto, Ont) 2022; 29(2): 698-723 [PMID: 35200560 PMCID: PMC8870737 DOI: 10.3390/curroncol29020061]
21. Tornesello ML, Buonaguro L, Tatangelo F, Botti G, Izzo F, Buonaguro FM. Mutations in TP53, CTNNB1 and PIK3CA genes in hepatocellular carcinoma associated with hepatitis B and hepatitis C virus infections. Genomics 2013; 102(2): 74-83 [PMID: 23583669 DOI: 10.1016/j.ygeno.2013.04.001]
22. Joseph NM, Tsokos CG, Umetsu SE, Shain AH, Kelley RK, Onodera C, Bowman S, Talevich E, Ferrell LD, Kakar S, Krings G. Genomic profiling of combined hepatocellular-cholangiocarcinoma reveals similar genetics to hepatocellular carcinoma. The Journal of pathology 2019; 248(2): 164-178 [PMID: 30690729 DOI: 10.1002/path.5243]
23. Pertea M. The human transcriptome: an unfinished story. Genes 2012; 3(3): 344-360 [PMID: 22916334 PMCID: PMC3422666 DOI: 10.3390/genes3030344]
24. Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szcześniak MW, Gaffney DJ, Elo LL, Zhang X, Mortazavi A. A survey of best practices for RNA-seq data analysis. Genome Biol 2016; 17(1): 13 [PMID: 26813401 PMCID: PMC4728800 DOI: 10.1186/s13059-016-0881-8]
25. Bayat A. Science, medicine, and the future: Bioinformatics. BMJ (Clinical research ed) 2002; 324(7344): 1018-1022 [PMID: 11976246 PMCID: PMC1122955 DOI: 10.1136/bmj.324.7344.1018]
26. O'Donoghue S. Grand Challenges in Bioinformatics Data Visualization. Front Bioinform 2021; 1: 669186 [PMID: 36303723 PMCID: PMC9581027 DOI: 10.3389/fbinf.2021.669186]
27. van Hal NL, Vorst O, van Houwelingen AM, Kok EJ, Peijnenburg A, Aharoni A, van Tunen AJ, Keijer J. The application of DNA microarrays in gene expression analysis. Journal of biotechnology 2000; 78(3): 271-280 [PMID: 10751688 DOI: 10.1016/s0168-1656(00)00204-2]
28. Sánchez-Peña ML, Isaza CE, Pérez-Morales J, Rodríguez-Padilla C, Castro JM, Cabrera-Ríos M. Identification of potential biomarkers from microarray experiments using multiple criteria optimization. Cancer medicine 2013; 2(2): 253-265 [PMID: 23634293 PMCID: PMC3639664 DOI: 10.1002/cam4.69]
29. Smyth GK. Limma: linear models for microarray data. Bioinformatics and computational biology solutions using R and Bioconductor: Springer, 2005: 397-420
30. Lombardo D, Saitta C, Giosa D, Di Tocco FC, Musolino C, Caminiti G, Chines V, Franzè MS, Alibrandi A, Navarra G, Raimondo G, Pollicino T. Frequency of somatic mutations in TERT promoter, TP53 and CTNNB1 genes in patients with hepatocellular carci-

noma from Southern Italy. Oncology letters 2020; 19(3): 2368-2374 [PMID: 32194736 PMCID: PMC7039085 DOI: 10.3892/ol.2020.11332]
31. Abdelsalam RA, El-Shawaf IM, Abdel-Aziz A, Bismar TA, Yussif SM. Wnt/β-catenin and CTNNB1 gene mutation in hepatocellular carcinoma, a case study in Egyptian patients. Surg Exp Pathol 2025; 8(1): 1 [DOI: 10.1186/s42047-025-00175-7]
32. Brandi G, Deiana C, Galvani L, Palloni A, Ricci AD, Rizzo A, Tavolari S. Are FGFR and IDH1-2 alterations a positive prognostic factor in intrahepatic cholangiocarcinoma? An unresolved issue. Front Oncol 2023; 13: 1137510 [PMID: 37168376 PMCID: PMC10164916 DOI: 10.3389/fonc.2023.1137510]

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Case Report

Liver Transplantation in a Patient with HIV and Hepatitis B Co-infection

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Abstract

HIV-infected individuals may develop co-infection and liver failure due to hepatitis viruses sharing similar transmission routes. While HIV infection was considered a contraindication for liver transplantation in the past, liver transplantation can be performed in these patients today as a result of the progress made in antiretroviral therapies. In this article, we described the liver transplantation procedure we performed on a patient infected with HIV and Hepatitis B virus and on antiretroviral therapy. Post-transplant follow-up and treatment of these patients should be performed carefully and meticulously by a multidisciplinary team.

Keywords: HIV, Hepatitis B, Liver Transplantation

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Before the advent of combined highly active antiretroviral therapy (cART), a positive HIV serological status was considered an absolute contraindication for solid organ transplantation.[1,2] However, the introduction of cART in 1996 significantly improved the life expectancy of HIV-positive individuals, subsequently increasing the prevalence of end-stage liver disease in this population.[2] In HIV-infected individuals, liver failure has become a common cause of death, particularly due to coinfection with hepatitis viruses (e.g., hepatitis B and C), which share similar transmission routes with HIV.[3] This has made liver transplantation (LT) an increasingly viable and necessary treatment option for HIV-positive patients. Today, LT in patients with HIV and hepatitis B coinfection can yield successful outcomes when approached with careful patient selection and a multidisciplinary strategy. This case report aims to shed light on the transplant process of a patient coinfected with HIV and hepatitis B, focusing on post-transplant management, challenges, and prognosis.

Case Report

Several precautions and preparatory steps were taken before and during the operation. A meticulous plan was implemented to minimize the risk of HIV transmission and to ensure safety throughout the procedure. The surgical team was thoroughly informed about HIV transmission routes and preventive measures, with special attention paid to avoiding contact with blood and body fluids. All surgeons

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and healthcare staff used double-layer sterile gloves, fluid-resistant gowns, protective eyewear (face shields or goggles), and masks. Special caution was taken while handling sharp instruments such as scalpels and needles, with disposable tools being preferred. The surgical field and all instruments were appropriately sterilized before and after the procedure. To reduce the risk of blood contamination, "hands-free" techniques were employed (e.g., avoiding direct hand-to-hand instrument exchange between the surgeon and assistant/nurse).

The case presented involves a 50-year-old male patient, 164 cm in height and weighing 104 kg, referred from another center due to the need for LT. The patient had no history or current signs of encephalopathy, ascites, or esophageal variceal bleeding. Abnormal laboratory findings included: hemoglobin: 9.7 g/dL; platelets: 212,000; INR: 1.6; creatinine: 1.4 mg/dL; albumin: 2.1 g/dL; total bilirubin: 5.9 mg/dL; alkaline phosphatase: 253 U/L; sodium: 131 mEq/L. MELD-Na score was 26. Tumor markers were within normal limits. ELISA results showed HBsAg: 1107 S/Co, Anti-HBc Total was positive, Anti-HBs was negative, Anti-HIV 263.5 S/Co, and HIV RNA was negative. The patient reported that he had been receiving regular antiretroviral therapy for HIV for 8 years.

After taking all necessary protective measures to prevent HIV transmission, a right lobe graft from an unrelated donor (approved by an independent ethics committee) was transplanted. The native liver appeared atrophic and nodular. The postoperative course of the patient was uneventful. Immunosuppressive treatment was based on tacrolimus and corticosteroids.

During the operation, a needle-stick injury occurred involving the second surgeon and a nurse. For these individuals, post-exposure prophylaxis was initiated immediately after low-to-moderate risk contact, with a one-month course of oral Emtricitabine (FTC) 200 mg + Tenofovir disoproxil fumarate 300 mg + Dolutegravir 50 mg. Anti-HIV tests repeated at the 6th week and 3rd month for both individuals were negative.

Discussion

Significant advances in liver transplantation (LT) for HIV-positive individuals have transformed what was once deemed a contraindication into a viable and increasingly successful therapeutic option. Recent studies demonstrate that LT outcomes in patients coinfected with HIV and hepatitis B virus (HBV) are comparable to those in HBV-monoinfected recipients, provided that perioperative and long-term management strategies are executed with precision.[2] In contrast, outcomes for those with HCV coinfection remain lower, although recent advances in HCV treatment offer promising improvements.[4,5]

A major challenge in such cases lies in the management of immunosuppressive therapy. Immunosuppressive therapy, essential to prevent organ rejection, increases the risk of opportunistic infections in HIV-positive patients due to immune suppression.[3] HIV-infected recipients are inherently immunocompromised, and the added burden of post-transplant immunosuppression requires a delicate balance between preventing graft rejection and minimizing the risk of opportunistic infections and HIV progression. Another critical issue is the potential interactions between immunosuppressive therapy and antiretroviral treatment. Immunosuppressive drugs may interact with antiretroviral medications, which can alter the response to treatment, increase drug toxicity, or reduce therapeutic effectiveness.[5] Calcineurin inhibitors—particularly tacrolimus—are commonly employed and generally well tolerated. However, when used concomitantly with antiretroviral therapy (ART), close monitoring becomes imperative. Notably, drug-drug interactions between immunosuppressants (e.g., tacrolimus, cyclosporine) and ART agents—especially protease inhibitors and certain integrase inhibitors—are mediated by cytochrome P450 enzymes, primarily CYP3A4. If unmanaged, these interactions can result in either toxic drug levels or subtherapeutic immunosuppression.

In our case, a post-transplant regimen of tacrolimus and corticosteroids was administered, alongside a stable ART combination of tenofovir, emtricitabine, and dolutegravir. This approach effectively mitigated interaction risks while maintaining immunological stability and viral suppression. Critically, the patient's HIV RNA remained undetectable—a prerequisite for transplantation according to current guidelines, which also recommend a CD4+ count above 100–200 cells/mm^3 for eligibility.[6,7]

In the context of HBV coinfection, lifelong antiviral prophylaxis is essential to prevent viral reactivation. Although our patient had elevated HBsAg and undetectable HBV DNA at the time of transplant, a tenofovir-based regimen was continued. The use of hepatitis B immune globulin (HBIG) remains optional in patients receiving potent antiviral therapy but may be considered in high-risk scenarios. Routine post-transplant HBV DNA monitoring is recommended, particularly in recipients receiving immunosuppressive agents known to promote HBV replication.

An additional concern is occupational exposure during surgery. Despite strict adherence to safety protocols, accidental needle-stick injuries occurred in our case. Immediate initiation of post-exposure prophylaxis (PEP) and structured follow-up prevented seroconversion, underscoring

the importance of institutional preparedness and continuous training for healthcare personnel involved in transplant procedures involving HIV-positive patients.

Ultimately, the successful management of such complex cases hinges on a multidisciplinary approach. Effective collaboration among hepatologists, infectious disease specialists, transplant surgeons, clinical pharmacists, and nursing staff is vital to navigate therapeutic complexities, minimize complications, and ensure favorable long-term outcomes.

Conclusion

In conclusion, liver transplantation is a viable and effective option for patients coinfected with HIV and hepatitis B, provided that HIV is well-controlled and a multidisciplinary approach is adopted. Careful coordination between transplant and infectious disease teams, along with vigilant post-transplant monitoring, is essential for success.

Key take-home points:

- LT is feasible in HIV-HBV coinfected patients with undetectable HIV viral load and adequate CD4+ count.
- Drug interactions between ART and immunosuppressants require close monitoring.
- Lifelong HBV prophylaxis and adherence to ART are critical.
- Multidisciplinary care improves long-term outcomes and reduces complications.

Disclosures

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

Conflict of Interest: None declared.

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References

1. Terrault NA, Roland ME, Schiano T, Dove L, Wong MT, Poordad F, et al. Outcomes of Liver Transplantation in HCV-HIV Coinfected Recipients. Liver Transpl. 2012 Jun;18(6):716–26.
2. Congly SE, Doucette KE, Coffin CS. Outcomes and management of viral hepatitis and human immunodeficiency virus coinfection in liver transplantation. World J Gastroenterol. 2014 Jan 14;20(2):414–24.
3. Tang J, Weng R, Fang T, Zhang K, Yan X, Jin X, et al. Clinical outcomes of liver transplantation in human immunodeficiency virus/hepatitis B virus coinfected patients in China. BMC Infect Dis. 2024 Apr 8;24:383.
4. Ince V, Ozdemir F, Bayindir Y, Toprak HI, Harputluoglu M, Kutlu R, et al. Liver Transplant in Patients with Viral Hepatitis and Human Immunodeficiency Virus Coinfection: The First 2 Cases in Turkey. Exp Clin Transplant. 2016 Jan 20.
5. Baccarani U, Righi E, Adani GL, Lorenzin D, Pasqualucci A, Bassetti M, et al. Pros and cons of liver transplantation in human immunodeficiency virus infected recipients. World J Gastroenterol. 2014 May 14;20(18):5353–62.
6. Roland ME, Barin B, Huprikar S, Murphy B, Hanto DW, Blumberg E, et al. Survival in HIV-positive transplant recipients compared with transplant candidates and with HIV-negative controls. AIDS. 2016 Jan 28;30(3):435–44.
7. Lynch EN, Russo FP. Liver Transplantation in People Living with HIV: Still an Experimental Procedure or Standard of Care? Life (Basel). 2023 Sep 27;13(10):1975.

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Erratum

In the article "*Carr B, Sotákov P, Pancoska P. A New Approach to Analysis of Clinical Data and Prognostication for Patients with Hepatocellular Carcinoma, Based Upon a Network Phenotyping Strategy (NPS) Computational Method.*" J Inonu Liver Transpl Inst 2024;2(3):109–116, published in the December 2(3) issue, the author order was incorrectly listed. The authors notified the editor of the issue, and upon evaluation, the editor approved the correction. The author order has been updated to: **Petr Pancoska[1], Patricia Sotákov[2], Brian I. Carr[3]**, and the article's PDF file has been revised accordingly.

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