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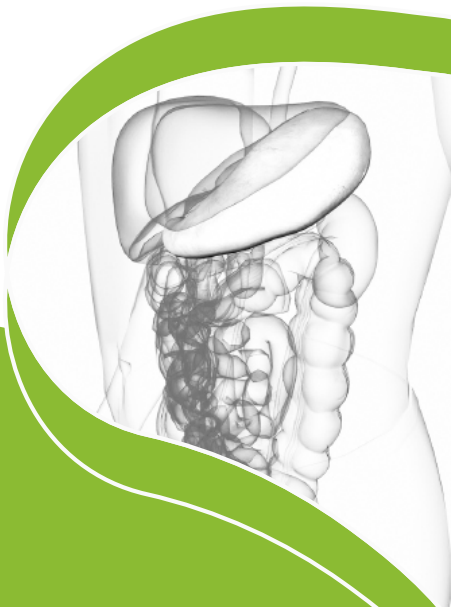

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AUTHOR STANDARDS **2025**

1. RAPD Objectives and characteristics: The Revista Andaluza de Patología Digestiva is the official publication of the Andalusian Society of Digestive Pathology (SAPD), which since 2007 has been published in electronic format only, under the name RAPD Online. Its purpose is to disseminate all epidemiological, clinical, basic and sociological aspects of digestive diseases, through the contributions sent to the journal from Andalusia and from the entire scientific community. The official language for the publication of this journal is Spanish, but some contributions may be accepted in the author's original language in English, French or Italian. RAPD Online is published bimonthly, with one of the issues being specially dedicated to the Annual Meeting of the SAPD and the Editorial Board deciding to reserve one or more issues per year for the monographic development of a topic related to the speciality.

All submitted contributions must be original and not be simultaneously under review for publication in another journal. The publication of abstracts or posters is not considered duplicate publication. Manuscripts will be evaluated by expert reviewers, appointed by the editorial board, before being accepted for publication, in a process that will take less than 30 days.

2. RAPD Contents: *regular numbers of RAPD Online include defined sections such as:*

- *Original articles on clinical or basic research.*
- *Thematic reviews on specific aspects of Gastroenterology.*
- *Consensus documents.*
- *Clinical cases.*
- *Clinical cases with videos or Videoforum.*
- *Images of the month.*
- *News and updates on gastroenterology and hepatology.*
- *Letters to the Editor.*

Other contributions that are considered of interest by the Editorial Board, relating to different aspects of clinical practice in the recent past, biographical comments, or other contents of a cultural nature, or related to scientific activities in any territorial area, will be inserted in RAPD Online in sections designed specifically for this purpose.

3.Submission of manuscripts: *The preferred way to submit manuscripts is through the SAPD website (https://www.sapd. es), by accessing the RAPD Online page and clicking on the "Submit an original" button located on the same access page to the journal. This will take you to the Manuscript Centre, from where you will be able to send manuscripts and all the required documentation. To use this tool you must be previously registered, access requires a username and password. If you are a member of the SAPD, you can use your usual username. If you are not a member, you can request a username for access to the Manuscript Centre using the form on the website. You can write to sulime@sulime.net or RAPDonline@sapd.es, for the solution of any problem in the submission of manuscripts.*

4. Writing standard for manuscripts: *monographic numbers, thematic reviews, updates and annotated articles will be commissioned by the Editorial Board, but the submission of any of these contributions at the request of an author will be considered by the RAPD Online Management and evaluated with great interest for inclusion in the journal.*

All manuscripts will be subject to specific rules, depending on the type of contribution, and to common ethical and legal standards.

A) Specific standard for manuscripts writing

They refer to the recommended length and structure of each type of manuscript. As a basic unit of length for the text, in any of the contributions, a page of 30-31 lines, spaced 1.5 lines apart, with a font size of 12, with 75-80 characters without spaces per line and a total of 400-450 words per page is considered. Texts should be sent spell-checked and in editable format in all their applications (main text, figures, legends or figure captions, tables, graphs, drawings).

Originals: *originals can be up to 12 pages long (5,100 words), excluding bibliographical references and captions to figures and tables. It is not advisable to insert more than 10 images, including tables and figures. Colour illustrations and videos will not represent an economic charge for the authors, but the insertion of videos, for technical reasons, will be previously agreed with the editor. However, the editing method of RAPD Online allows, in specific cases, the acceptance of longer manuscripts, or the inclusion of a greater number of images, provided that the characteristics of the material presented so require. It is not advisable to have more than 9 authors, except in the case of collaborative works. In these originals, the first nine participants will be listed at the head of the paper and the rest of the participants will be listed at the end of the first page of the manuscript.*

Through the Manuscript Centre, the following information will be required for the submission of an original:

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4° Full postal address of the responsible author, to whom correspondence should be addressed, including telephone, fax and email address.
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- Main body of the manuscript, containing:

1° Structured abstract in Spanish (optional also in English) and 3-5 keywords. The abstract will have a maximum length of 250 words and should be structured as follows:

a) Introduction and objectives
b) Material and methods
c) Results
d) Conclusions

2° List of abbreviations used in the text.

3° Text: it will include the following sections:
a) Introduction
b) Material and methods
c) Results

d) Discussion
e) Conclusions; each of them appropriately headed.

4° Bibliography: according to the specifications established in the group of common standards (See common standards and other supporting documents).

5° Acknowledgements.

6° Figure captions.

7° Tables and figures in text.

Thematic Reviews: *texts on Thematic Reviews can be up to 15 pages long (6,375 words), excluding bibliographical references and captions to figures and tables, and chapters corresponding to Update series up to 20 pages (8,500 words). In both cases the number of inserted images should not exceed 15, including tables and figures. However, the RAPD Online editing method allows, in specific cases, for manuscripts of greater length, or the inclusion of a greater number of images, provided that the characteristics of the material presented so require. Illustrations in colour will not be charged to the authors. Exceptionally, the inclusion of videos will be accepted. It is not advisable to include more than 4 authors per chapter.*

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- Main body of the manuscript, containing:

1° Structured abstract in Spanish and English. 3-5 key words. The abstract will have a maximum length of 350 words, emphasising the most important aspects of the manuscript.
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5° Declaration on the existence or non-existence of a source of funding for the work, or conflicts of interest.

- Main body of the manuscript, containing:

1° Structured abstract in Spanish and English. 3-5 key words. The abstract will have a maximum length of 350 words, emphasising the most important aspects of the manuscript.
2° Text: Structured according to the criteria of the author(s), for a better understanding of the topic developed.
3° Bibliography: According to the specifications established in the group of common standards (See common standards and other supporting documents).
4°Acknowledgements
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6° Tables and Figures in the text.

Clinical Cases: *the manuscripts included in this section will include 1-5 clinical cases, which due to their infrequent or unusual clinical behaviour, or because they provide some diagnostic or therapeutic novelty, deserve to be reported.*

The length of the texts in the Clinical Cases section should not exceed 5 pages (2,125 words), excluding bibliographical references and captions to figures and tables, and the number of inserted images should not exceed 5, including tables and figures. However, the RAPD Online editing method allows, in specific cases, the acceptance of longer manuscripts, or the inclusion of a greater number of images, provided that the characteristics of the material presented so require. Colour illustrations and videos will not represent a financial charge for authors, but the insertion of videos, for technical reasons, will be previously agreed with the editor. No more than 5 authors will be admitted, except in specific and reasoned cases.

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4° Full postal address of the responsible author, to whom correspondence should be addressed, including telephone, fax and e-mail address.

- Main body of the manuscript, containing:

1° Structured abstract in Spanish and English. 3-5 key words. The abstract will have a maximum length of 250 words.
2° Introduction. To present the clinical problem reported.
3° Description of the clinical case.
4° Discussion. To highlight the peculiarities of the case and its consequences.
5° Bibliography: According to the specifications established in the group of common standards (See common standards and other supporting documents).
6°Acknowledgements.7° Figure captions.
8° Tables and text figures.

Clinical Cases with Videos or Videoforum: *the manuscripts included in this section will include 1-5 clinical cases, which due to their infrequent or unusual clinical behaviour, or because they provide some diagnostic or therapeutic novelty, deserve to be communicated.*
The length of the texts in the Videoforum section should not exceed 5 pages (2,125 words), excluding bibliographical references and captions to figures and tables, and the number of images inserted should not exceed 5, including tables and figures. However, the RAPD Online editing method allows, in specific cases, the acceptance of longer manuscripts, or the inclusion of a greater number of images, provided that the characteristics of the material presented so require. Colour illustrations and videos will not represent a financial charge for authors, but the insertion of videos, for technical reasons, will be previously agreed with the editor. No more than 5 authors will be admitted, except in specific and reasoned cases.

Videos should be submitted in AVI, MPEG, MP4 OR MOV format, and at a recommended high quality resolution (720p or 1080p). They must not contain personal data of the patients. It is recommended that they be edited to minimise editing time, which should not exceed 10 minutes. If the video includes sound, it must be processed in MP3 format. If the videos to be included are in other formats, please contact the publisher

to verify their validity. They should not exceed 2GB.
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3° Centre(s) of origin (department, institution, city and country).
4° Full postal address of the responsible author, to whom correspondence should be addressed, including telephone, fax and email address.

- Main body of the manuscript, containing:

1° Structured abstract in Spanish and English. 3-5 key words. The abstract will have a maximum length of 250 words.
2° Introduction. To present the clinical problem reported. 3° Description of the clinical case.
4° Discussion. To highlight the peculiarities of the case and its consequences.
5° Bibliography: According to the specifications established in the group of common standards (See common standards and other supporting documents).
6°Acknowledgements.7° Figure captions.
8° Tables and figures in text.
9° Videos.

Link tutorial videos: https://www.sapd.es/videoteca/varios/ tutoriales/

Images of the month: *the manuscripts included in this section can take two formats, depending on the authors' preference.*

- Format A. Images with educational value: these shall include images of any kind, clinical, radiological, endoscopic, anatomopathological, macro and microscopic, which contribute to postgraduate training and therefore deserve to be shown because of their peculiarity, or because they represent a characteristic example.
- Format B. Key images for a diagnosis: These will include images of any kind, clinical, radiological, endoscopic, anatomopathological, macro and microscopic, together with a summarised clinical history, which will provide the possible final diagnostic resolution. This will be presented in a separate section in the same issue of the journal.

The length of the texts in the Images of the Month section must not exceed 1 page (425 words) in the clinical approach to the image presented and 2 pages (850 words), excluding bibliographical references and captions to figures and tables, in the commentary on the image (Format A) or in the diagnostic resolution of the case (Format B). However, the RAPD Online editing method allows, in specific cases, the acceptance of longer manuscripts, or the inclusion of a greater number of images, provided that the characteristics of the material presented so require. Colour illustrations and videos will not represent a financial charge for authors, but the insertion of videos, for technical reasons, will be previously agreed with the editor. No more than 3 authors will be accepted, except in specific and reasoned cases.

Through the Manuscript Centre, and for the submission of an Image of the Month, the following information will be required:

-General data:

1° Full title of the work in Spanish and English.. 2° Surnames and first names of all authors.
3° Centre(s) of origin (department, institution, city and country).
4° Full postal address of the responsible author, to whom correspondence should be addressed, including telephone, fax and email address.
5° Type of Image of the Month format chosen.

-Main body of the manuscript, containing:

1° Structured abstract in Spanish and English and 3-5 key words. The abstract will have a maximum length of 250 words.
2° Description of the image.
3° Comments on the image.
4° Bibliography: According to the specifications established in the group of common standards (See common standards and other supporting documents).
5° Figure captions.

New developments and updates in gastroenterology and hepatology: this section will be devoted to commenting on the scientific and medical developments that have occurred in recent years in the speciality of Gastroenterology and Hepatology.

This section will systematically and periodically analyse all facets of the speciality.

Texts on " New developments in Gastroenterology" may be up to 5 pages long (2,125 words), excluding bibliographical references and captions to added figures and tables. In both cases the number of inserted images must not exceed 5, including tables and figures. However, the RAPD Online editing method allows,

in specific cases, the acceptance of longer manuscripts, or the inclusion of a greater number of images, provided that the characteristics of the material presented so require. It is not advisable to have more than 3 authors per chapter.

Through the Manuscript Centre, you will be asked to provide the following information:

- General data:

1° Name of the bibliographic area reviewed and period analysed 2° Surname and first name of all authors. It is advisable to place a hyphen between the first and second surname.
3° Centre(s) of origin (department, institution, city and country).
4° Full postal address of the responsible author, to whom correspondence should be addressed, including telephone, fax and email address.
5° Declaration on the existence or non-existence of a source of funding for the work, or conflicts of interest.

- Main body of the manuscript, containing:

1° Structured abstract in Spanish and English. 3-5 key words. The abstract will have a maximum length of 250 words.
2° Description of the bibliographic material analysed.
3° Critical comments on the results contained in the selected works.
4° Bibliography: According to the specifications established in the group of common standards (See common standards and other supporting documents). If two or more originals have been chosen for the analysis, it is advisable to divide the section into sections at the authors' discretion.
5° Figure captions.
6° Tables and Figures in text.

Letters to the Editor: *this section will be dedicated to comments on any manuscript published in RAPD Online. This section may also include comments of a more general nature, establishing the authors' own hypotheses and suggestions, within the scientific field of Gastroenterology. The length of the texts in this section of Letters to the Editor should not exceed 2 pages (850 words), including bibliographical references. Two figures or tables may be included and the number of authors should not exceed four.*

Through the Manuscript Centre, and for the submission of a Letter to the Editor, the following information will be required:

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2° Surnames and first names of all authors. It is advisable to place a hyphen between the first and second surname.
3° Centre(s) of origin (department, institution, city and country).
4° Full postal address of the responsible author, to whom correspondence should be addressed, including telephone, fax and e-mail address.
5° Declaration on the existence or non-existence of a source of funding for the work, or conflicts of interest.

- Basic body of the manuscript, containing:

1° Text of the manuscript.
2° Bibliography: According to the specifications set out in the common standards group (See common standards and other supporting documents).

B)Common standards and other supporting documents

This refers to the set of mandatory standards, both for uniformity in the presentation of manuscripts and for compliance with current legal regulations. In general, the style of manuscripts should follow the guidelines set out in the Vancouver Agreement of the International Committee of Medical Journal Editors. (http://www.ICMJE.org).

Units, generic names and abbreviations:

- Units. Biochemical and haematological parameters shall be expressed in International Units (SI), except haemoglobin which shall be expressed in g/dL. Length, height and weight measurements shall be expressed in decimal metric units and temperatures in degrees Celsius. Blood pressure shall be measured in millimetres of mercury.
There is an aid for the conversion of non-international (non-SI) units into international (SI) units. (http://www.techexpo.com/techdata/techcntr.html).

- Generic names. The generic names of medicinal products, clinical instruments and tools and software shall be used. When a brand name is the subject of research, the brand name and the name of the manufacturer, city and country shall be included in parentheses the first time the generic name is mentioned in the Methods section.

- Abbreviations. Abbreviations should be avoided, but if they have to be used, in order not to repeat long technical names, the full word should appear the first time in the text, followed by the abbreviation in brackets, which will already be used in the manuscript.

Bibliographical references: bibliographical references should be presented in the order in which they appear in the manuscript, with a sequential number, which will appear in the appropriate place in the text, in brackets. This numbering will be maintained and will serve to order the list of all references at the end of the manuscript, as normal text and never as a footnote. Personal communications and unpublished data will not be included in the final list of bibliographical references, although they will be mentioned in the appropriate place in the text, in brackets, as appropriate, i.e. personal communication or unpublished data. When the bibliographic citation includes more than 6 authors, the first 6 authors should be cited, followed by the abbreviation et al.

The style of bibliographic references will depend on the type and format of the source cited:

- Medical journal article: journal names should be abbreviated according to the style of the Index. Medicus/Medline (http://www.ncbi.nlm.nih.gov/journals?itool=sidebar).

- Article already published in print and online journals Internet:
The authors (surname and initial of the first name, comma separation between authors), the full name of the manuscript, the abbreviation of the journal, the year of publication and after a semicolon the volume of the journal and after a colon the complete numbers of the first and last page of the paper.

Kandulsky A, Selgras M, Malfertheiner P. Helicobacter pylori infection: A Clinical Overview. Dig Liver Dis 2008; 40:619-626.

Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK,Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999; 31:929-938.

- Admitted article, published only on the Internet, but not yet included in a regular number of the journal: the authors, the full name of the manuscript, the abbreviation of the journal, the year and month since the article is available on the Internet and DOI will be noted. The original paper to which reference is made usually details how to cite the manuscript.

Stamatakos M, Sargedi C, Stefanaki C, Safi oleas C, Matthaiopoulou I, Safi oleas M. Anthelminthic treatment: An adjuvant therapeuticstrategy against Echinococcus granulosus. Parasitol Int (2009), doi:10.1016/j.parint.2009.01.002

Inadomi JM, Somsouk M, Madanick RD, Thomas JP, Shaheen NJ. A cost-utility analysis of ablative therapy for Barrett'sesophagus, Gastroenterology (2009), doi: 10.1053/j.gastro.2009.02.062.

- Article from a journal that is published only on the Internet, but arranged in a conventional way: the authors, the full name of the manuscript, the abbreviation of the journal (can be added in brackets online), the year of publication and after a semicolon the volume of the journal and after a colon the complete numbers of the first and last page of the paper. If the original work being referenced provides the DOI and Internet address (URL), these can be added at the end of the reference.

Gurbulak B, Kabul E, Dural C, Citlak G, Yanar H,Gulluoglu M, et al. Heterotopic pancreas as a leading point for small-bowel intussusception in a pregnant woman. JOP (Online) 2007; 8:584-587.

Fishman DS, Tarnasky PR, Patel SN, Raijman I. Managementof pancreaticobiliary disease using a new intra-ductal endoscope: The Texas experience. World J Gastroenterol 2009; 15:1353-1358. Available from: URL: http://www.wjgnet.com/1007-9327/15/1353.asp. DOI: http://dx.doi.org/10.3748/wjg.15.1353

- Article from a journal that is published only on the Internet, but is not conventionally arranged: the authors, the full name of the manuscript, the abbreviation of the journal, the year of publication and the DOI will be listed.

Rossi CP, Hanauer SB, Tomasevic R, Hunter JO, Shafran I, Graffner H. Interferon beta-1a for the maintenance of remission in patients with Crohn's disease: results of a phase II dose-finding study.BMC Gastroenterology 2009, 9:22doi:10.1186/1471-230X-9-22.

-Article published in an abstract or in a supplement of a journal: the authors (surname and first initial, separated by commas between authors), the full name of the manuscript, the word abstract in square brackets, the abbreviation of the journal, the year of publication and after a semicolon the volume of the journal, followed by the abbreviation Suppl, or Supl, in brackets and after a colon the complete numbers of the first and last page of the paper.

Klin M, Kaplowitz N. Differential susceptibility of hepatocytesto TNF-induced apoptosis vs necrosis [Abstract]. Hepatology 1998; 28(Suppl):310A.

- Books: the authors of the book (surname and first initial, comma separated by the authors), the title of the book, the city where it was published, the name of the publisher and the year of publication will be listed.

Takada T. Medical Guideline of Acute Cholangitis and Cholecystitis. Tokyo: Igaku Tosho Shuppan Co; 2005.

- Chapter of a book: the authors of the chapter should be listed (surname and first initial, separated by commas between authors), followed by In: the names of the editors of the book and, after a full stop, the name of the book. The city where it was published, the name of the publisher, the year of publication and after a colon the complete numbers of the first and last page of the work.

Siewert JR. Introduction. In: Giuli R, Siewert JR, Couturier D, Scarpignato C, eds. OESO Barrett's Esophagus. 250 Questions. Paris: Hors Collection, 2003; 1–3.

- Information from a document produced at a meeting: this type of reference should be avoided whenever possible. However, if it has to be cited, the title of the subject, the name of the meeting and the city where it was held should be given. The entity that organised the meeting, and the year. The e-mail address through which the document can be accessed.

U.S. positions on selected issues at the third negotiating session of the Framework Convention on Tobacco Control. Washington, D.C.: Committee on Government Reform, 2002. (Accessed March 4, 2002, at:http://www.house.gov/reform/min/inves_tobacco/index_accord. htm.)

Figures, tables and videos: iconography, whether photographs, radiographs, diagrams or graphs, should be referred to under the generic name of "Figure". References to figures, tables and videos should be highlighted in bold. They should be numbered with Arabic numerals, according to their order of appearance in the text. Panels of two or more photographs grouped together will be considered as a single figure and may be referred to as "Figures 1A, 1B, 1C".

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Endoscopic images and other techniques generating colour images, if not directly available in electronic format, shall be scanned in colour. Special details shall be indicated by arrows, using for these and any other type of symbols the maximum contrast plot with respect to the figure.

Figure files will be identified according to their order of appearance in the text, with the name of the file, its number and surname of the first signatory (e.g.: fig1_Gómez.jpg) or the title of the article. Each image must have an associated figure caption that serves as a description. Figure captions must be submitted in a separate text document with clear reference to the figures to which they refer. Images may be inserted in Word/PowerPoint files to facilitate their identification or association with the figure captions, but they must always be submitted as separate images in the formats mentioned above.

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Human research: publications on human research should show in a prominent place in the original paper that: a) written informed consent has been obtained from each patient, b) The study protocol conforms to the ethical standards of the Helsinki Declaration of 1975 and has been approved by the ethical committee of the institution where the study was conducted.
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Data obtained by microarray: data must be sent to a repository such as Gene Expression Omnibus or ArrayExpress before submission of the manuscript.

Data protection
The personal data requested from the authors will be used by the Andalusian Society of Digestive Pathology (SAPD), exclusively for the purpose of managing the publication of the article submitted by the authors and accepted in the RAPD online. Unless otherwise indicated, by submitting the article the authors expressly authorise that their data relating to name, surname, institutional postal address and e-mail address be published in the RAPD online, possibly in the annual abstracts published by the SAPD on CD support, as well as on the SAPD website and in Medline, or other bibliographic search agencies, to which the RAPD online may have access.

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- The statistical methods used, as well as the software and the name of the software used must be clearly stated in the Material and Methods Section.
-To express mean, standard deviation and standard error, the following formats must be used: "mean (SD)" and "mean ± SE". To express the median, the values of the interquartile range (IQR) must be used.
- The P must be used in capital letters, reflecting the exact value and expressions such as less than 0.05, or less than 0.0001 should be avoided.
- Whenever possible, findings (means, percentages, odds ratios and others) should be quantified and presented with appropriate error indicators, such as confidence intervals.
- Studies showing levels of statistical significance should include the calculation of sample size. Authors should state research losses, such as dropouts from clinical trials.

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ORIGINAL

TETRASPANINS AND miRNAs IN URINARY EXTRACELLULAR VESICLES IN PATIENTS WITH COLONIC POLYPS AND A FAMILY HISTORY OF COLORECTAL CANCER

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Abstract

Introduction: Urinary microRNAs (miRNAs) and extracellular vesicles (EVs) may serve as useful biomarkers for neoplastic (CRC) or preneoplastic colorectal lesions.

Patients and Methods: A prospective observational study was conducted on individuals with first-degree family relatives (FDR) of colorectal cancer (CRC) and a control group. Flow cytometry (FC) and next-generation sequencing were used to study the profile of tetraspanins and miRNAs carried by EVs in urine samples.

Results: A total of 46 individuals were included (mean age 53.52 ±7.71 years). Of these, 69.39% had a FDR CRC (group 1: 20 patients with hyperplastic polyps or no polyps, and group 2: 11 with adenomas). Fifteen individuals (group 3: controls) had neither FDR CRC nor polyps. Eighteen urine samples were analyzed. Two miRNAs (miR-141-3p and miR-30d-5p) were differentially expressed ($p<0.05$) in subjects with CRC FDR compared to controls. The tetraspanin profile (CD9, CD63, and CD81) of subjects with FDR CRC differed from that of controls. Quantitative PCR analysis confirmed the differences found in next-generation sequencing when comparing all groups.

Conclusions: The tetraspanin profile of individuals with FDR CRC differs from that of controls. This allows for the hypothesis that certain tetraspanins may serve as "biomarker

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of biomarkers." On the other hand, next-generation sequencing of miRNAs present in urinary EV samples enables the identification of their expression levels, thus representing a valid and non-invasive method for studying and monitoring their expression in different groups.

Keywords: Colorectal cancer, microRNA, extracellular vesicles, tetraspanins, next-generation sequencing, flow cytometry.

List of abbreviations

- FH CRC: family history of colorectal cancer.
- CRC: colorectal cancer.
- FC: flow cytometry.
- SD: standard deviation.
- miRNA: microRNA.
- qPCR: quantitative real-time PCR.
- IQR: interquartile range.
- EVs: extracellular vesicles.

Introduction

Colorectal cancer (CRC) ranks third in incidence and mortality in both sexe[1]. Thanks to early diagnosis and the implementation of population screening programs, mortality has decreased. However, due to lifestyle habits and lack of resources in health systems, CRC remains a major public health problem. Although most CRC are sporadic, about 15% have a family history of CRC (FH CRC)[2]. Therefore, early diagnosis of preneoplastic lesions is mandatory in individuals with first-degree FH CRC.

A current trend is the search for non-invasive tests aimed at diagnosing patients with precursor lesions or stratifying risk groups. These include biomarkers (liquid biopsy) that can be analyzed in a multitude of biological fluids. Urine is an excellent sample because of its ease of collection and storage and its abundance of metabolites and other molecules. One way to approach this type of research is the study of extracellular vesicles (EVs) and the molecules they carry. EVs are very abundant in the organism and constitute intercellular information vehicles[3,4].

Within the cargo of EVs, microRNAs or miRNAs and some membrane proteins, tetraspanins, stand out as markers of EVs, since in addition to being part of the membrane structure, they participate in the genesis, distribution, transport and elimination of the components of the cargo[5-8].

A multitude of miRNAs have been identified that appear to be involved in the development of polyps and CRC[9]. The miRNAs may have oncogenic (onco-miRNAs) or anti-oncogenic (anti-oncomiRNAs) activity[10-12]. Onco-miRNAs include: miR-31 (KRAS stimulator), miR-21, with oncogenic properties through repression of the PDCD4 (Programmed Cell Death 4) target (proinflammatory tumor suppressor), and miR-200, important in the maintenance of epithelial identity that represses promesenchymal transcription factors and promotes metastasis. On the other hand, among the miRNAs with anti-oncogenic functions, the following stand out: the miR-34 family, key in the repression of tumor migration, invasion and metastasis formation, and the miR let-7 family, included among the most important in the repression of oncogenesis due to its abundance and antiproliferative function.

Compared to liquid biopsy, urine is an excellent biological fluid that offers important advantages for its analysis: it is an affordable and convenient sample for the researcher and the patient, reproducible, little studied and abundant in EVs and miRNAs from different origins of the organism, including the colon.

The objectives of this work were to analyze the tetraspanin profile by flow cytometry (FC), to establish urinary miRNAs that could be useful as biomarkers of neoplastic or preneoplastic colonic pathology, and to demonstrate the practical value of urine in the analysis of EVs and intravesicular cargo.

Patients and methods

This was a prospective analytical observational study involving subjects undergoing colonoscopy for AF first-degree CRC aged 18-70 years and subjects who underwent screening colonoscopy (control group). The study was conducted between November 2019 and November 2020. All patients had a urine sample collected on the day of colonoscopy.

Exclusion criteria were: age <18 years or >70 years and persons with endoscopic findings of infectious/ischemic colitis, inflammatory bowel disease, or CRC.

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The colonoscopies had to meet the recommended quality criteria. The resected polyps were sent to the Anatomic Pathology service of each center..

On the day of the colonoscopy, a urine sample (10-20 ml) was extracted and stored in a freezer at -80°C until analysis. Subsequently, the samples were sent to the laboratory of the Applied Bioscience Techniques Service of the University of Extremadura for analysis by CF and massive sequencing. The selected samples were identified by a numerical code.

The data of each patient were archived with appropriate security measures in compliance with the Organic Law 15/1999 on Data Protection.

Statistical analysis

Qualitative variables were presented using frequency tables and quantitative variables were summarized as mean and standard deviation (SD) or median and interquartile range (IQR) for variables that did not follow a normal distribution. IBM SPSS© v. 20.0.0 was used.

Laboratory methodology and bioinformatic analysis

Urinary samples were centrifuged and filtered to obtain EVs making two aliquots: one for the study of tetraspanins by CF analysis and the other for the extraction of miRNAs and their massive sequencing using Ion Torrent technology.

For the study of tetraspanins in EV membranes, staining with Carboxyfluorescein Succinimidyl Ester (CSFE) was performed to analyze the quality of the preparation and the other sample was used for staining with the antiCD9, antiCD63 and antiCD81 antibodies conjugated with the different fluorochromes and thus visualized by the corresponding channels of the flow cytometer (Cytoflex S, BeckmanCoulter).

For the sequencing of miRNAs, we first extracted the small RNA population with the EasyPure miRNA kit (Transgenbiotech, China). The obtained miRNAs were measured with Agilent Bioanalyzer with the Bioanalyzer High Sensitivity RNA Analysis kit, (Agilent, USA). After checking that all the quality parameters were good, the preparation of the library for massive sequencing of the Ion Torrent platform was continued following the protocol of the Qiaseq miRNA Library Kit (Qiagen, Germany) with subsequent tempering in the IonChef (ThermoFisher, USA) with the Ion 550™ Kit-Chef kit (ThermoFisher, USA). Once the chip was loaded, it was read in the Ion S5 XL sequencer with the specific reagents included in the kit.

After sequencing the library, the miRNA sequences of each sample were obtained and used to search the following databases: miRBase v22genome-build-id: GRCh38, genome-build-accession: NCBI_Assembly: GCA_000001405.15), Bsgnenome.Hsapiens.UCSC.hg38. masked,https://bioconductor.org/packages/release/data/annotation/html/BSgenome.Hsapiens.UCSC.hg38.masked.html (reference genome) in order to identify which miRNAs were present in each sample and how many were copies. Subsequently, a bioinformatic analysis was performed to compare the miRNAs present in each group to identify those miRNAs that provided information on the prediction of the appearance of colorectal lesions.

Once the miRNAs of interest for their overexpression or underexpression were identified, Taqman probes were designed for each of them compatible for ThermoFisher cDNA Taqman Advanced miRNA Chef chemistry (ThermoFisher, USA) which was subsequently used to test whether the same information could be obtained by real-time quantitative PCR (qPCR), since this technique is faster and cheaper. Therefore, from the preparation of urinary EVs used for the massive sequencing study, new miRNA extraction was performed with the same protocol as the one described for sequencing. With the pool of miRNAs obtained and using TaqMan probes, qPCR was performed on a QuantStudio 6 thermal cycler (ThermoFisher, USA) following the protocol described for the kit.

Ethical considerations

Patients were informed verbally and in writing of the nature of the study and signed the informed consent. The study was approved by the Clinical Research Ethics Committee (CEIC) of the University Hospital of Badajoz.

Results

Initially, 46 subjects were included in the study with the collection of 46 urine samples, but, due to the Covid-19 pandemic situation, only 18 samples could be analyzed, which were considered sufficient in the investigation as proof of concept. The baseline characteristics of the patients, their lifestyle and history are shown in table 1. Of the 46 subjects, 31 (69.39%) had first-degree AF CRC, 20 of them (43.47%) had no polyps or hyperplastic polyps (group 1), 11 (23.91%) had colorectal adenomas (group 2) and the rest, 15 subjects (32.60%) who were the controls, had no history of first-degree CRC and no polyps.

All colonoscopies were complete, meeting quality standards. The preparation was adequate (Boston ≥ 6) in 43

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Sex	M: 30 (65.2%) F: 16 (34.8%)
Age	53.52 ± 7.71 years
Lifestyle habits:	
·Consumption of fiber, fruits, and vegetables	33 (71.1%)
·Consumption of red meat	21 (45.7%)
·Regular physical activity	20 (43.5%)
Smokers	9 (19.6%)
Alcohol consumption	11 (23.9%)
NSAIDs/ASA use	4 (26.1%)
Family history of other tumors	10 (2 cáncer gástrico)
FA: family history	

Table 1. ***Baseline characteristics, life habits, Baseline characteristics, life habits, consumption of toxins and drugs and family history of tumors of the study subjects.***

patients (93.48%). Regarding the histology of the adenomas, only one was advanced (high-grade dysplasia).

Analysis of tetraspanins

Table 2 shows the values described as percentage of positivity of CD9, CD81 and CD63 tetraspanins and figure 1 shows the comparison between the three groups.

Groups	CD9	CD81	CD63
Group 1	39.87 ± 6.11	66.30 ± 6.87	40.36 ± 4.01
Group 2	57.44 ± 5.44	78.75 ± 7.87	94.07 ± 3.99
Group 3	83.56 ± 7.89	79.17 ± 6.71	87.66 ± 8.45

Table 2. ***Percentage of positivity of tetraspanins CD9, CD81 and CD63 expressed with their mean±DE in each group.***

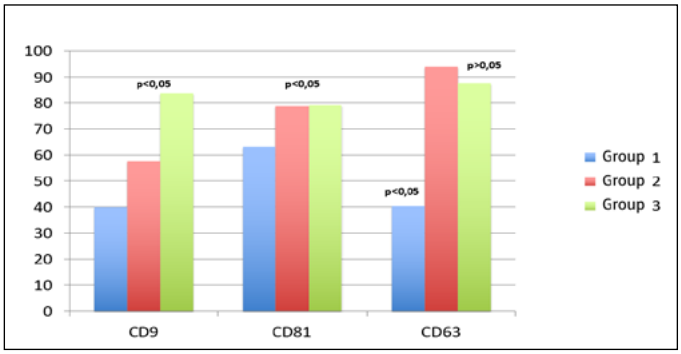

Figure 1. ***Comparison of tetraspanin CD9, CD81 and CD63 tetraspanin values between the 3 groups.***

CD9: lower values were observed in both groups with AF CRC compared to controls, with statistically significant differences ($p<0.05$).

CD81: lower levels were identified in both groups with AF CCR (groups 1 and 2) compared to controls.

CD63: levels in group 1 were lower than controls, with statistically significant differences ($p<0.05$). In group 2, levels were higher than in controls, although without statistical differences.

Analysis of microRNAs in urinary EVs

For the analysis of miRNAs present in urinary EVs, after alignment of the obtained sequences with published miRNA databases, comparison between groups was performed and represented in volcano plots (Figure 2). When comparing group 1 and group 3, 6 differentially expressed miRNAs were detected. Of note were hsa-mir-141-3p, down-expressed and with $p<0.05$, miR-let-7b, which is an oncogenic suppressor miRNA, also down-expressed and hsa-miR-10a-5p which is an oncogenic miRNA and found to be over-expressed.

Comparison between group 2 and group 3 allowed the detection of 4 differentially expressed miRNAs. Only hsa-miR-30d-5p showed a statistically significant elevated expression ($p<0.05$). The rest of miRNAs showed decreased expression in the control group of patients, with the hsa-miR-200a-3p oncomiRNA standing out.

An additional study was performed comparing both groups with AF CRC, finding hsa-miR-92-3p overexpressed in group 2. This same miRNA was also detected overexpressed when compared to controls.

Of all the miRNAs sequenced, only 9 showed significant differences between groups (hsa-miR-92a-3p, hsa-miR-200a-3p, hsa-miR-141-3p, hsa-miR-203a-3p, hsa-miR-375-3p, hsa-miR-10a-5p, hsa-miR-30d-5p, hsa-miR-888-5p and hsa-let-7b-5). These were selected for study by qPCR using Taqman probes specific for each (Figure 3). As normalizing or "housekeeping" genes, hsa-miR-186-5p and hsa-miR-191-5p were used, since they were present in all the samples in the massive sequencing and their expression levels were maintained equally in all of them regardless of the group. The differences found in the bulk sequencing were confirmed, i.e., in the cases in which a decrease or increase of that miRNA was identified, a decrease or increase in the expression by qPCR was seen.

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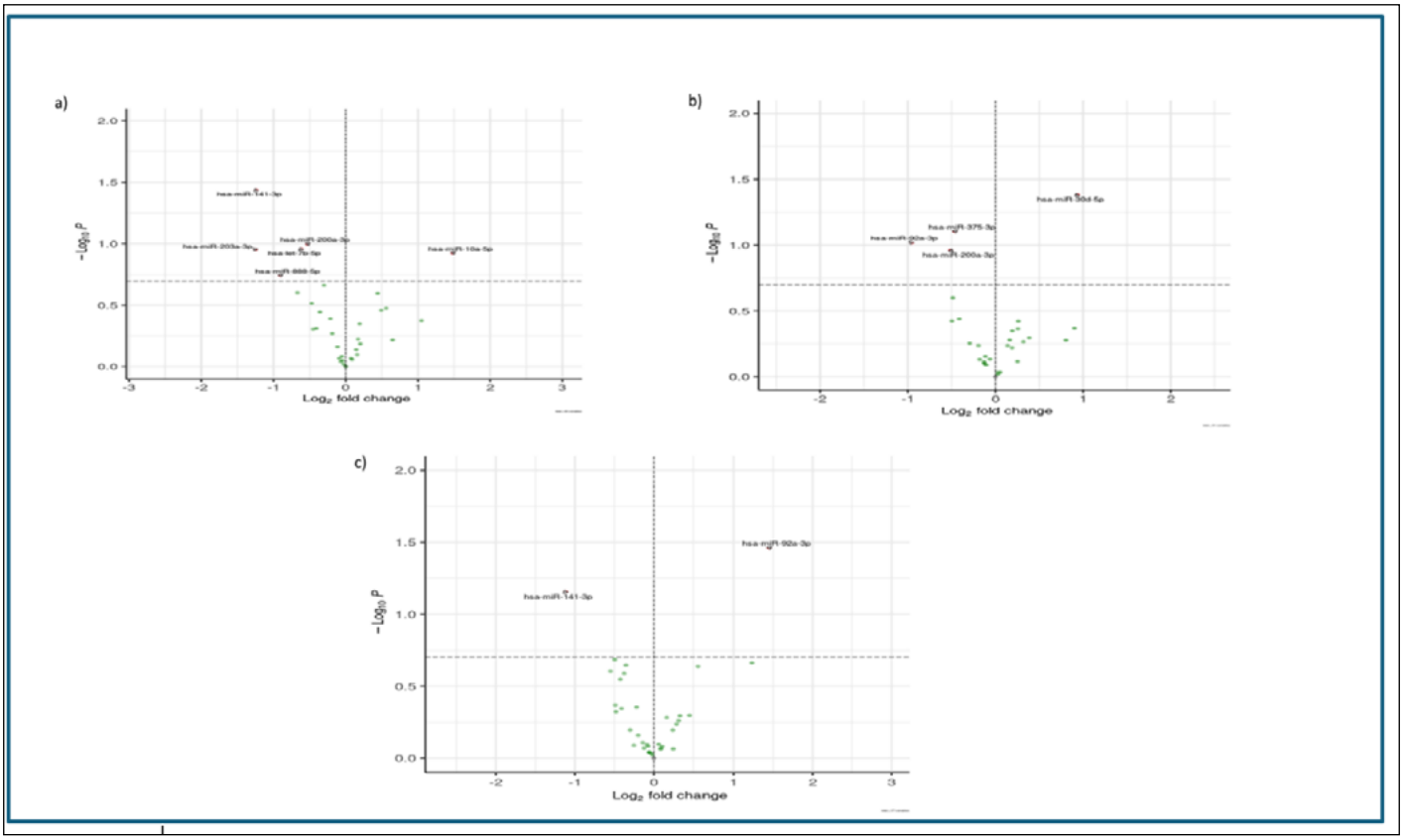

Figure 2. ***Volcano plots. a) Comparison between Volcano plots. a) Comparison between group 1 and group 3. b) Comparison between group 2 and group 3. c) Comparison between group 2 and group 1.***

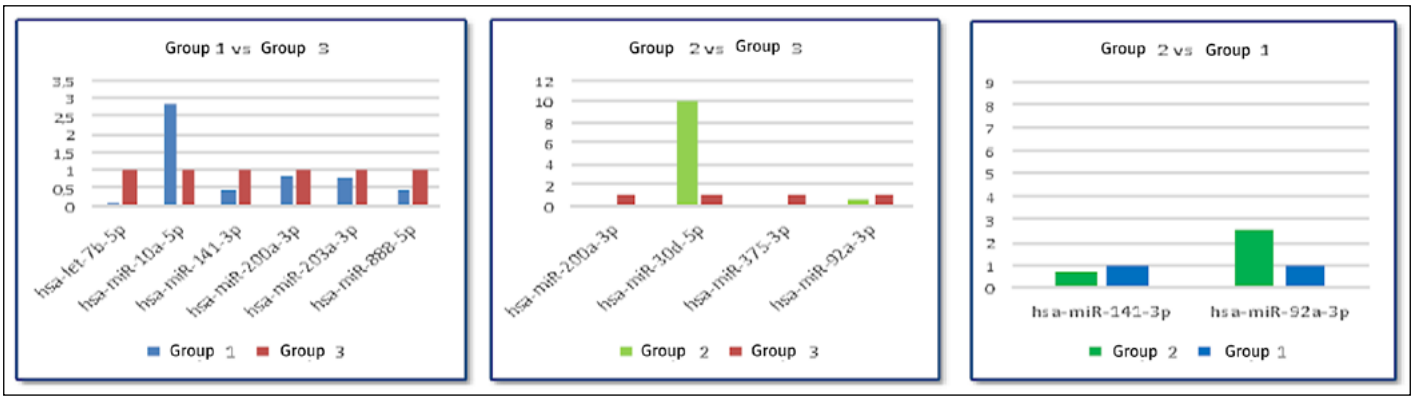

Figure 3. ***Validation of miRNAs expression Validation of miRNAs expression by qPCR.***

Discussion

In the present investigation we analyzed miRNAs carried in urine EVs, whose function is to regulate genomic transcription. A noteworthy aspect is the role of some miRNAs in the pathogenesis of adenomas and CRC. There is a growing literature on miRNAs detected in various types of biological samples. For example, in blood, miR-21, miR-92a, and miR-17-3p[13]; in tissues, an increase of 6 miRNAs from normal tissue to CRC: miR-18a, miR-18b, miR-431, miR-503, miR-1246, and miR-4417 and a decrease of 5 other miRNAs: miR-133a, miR-375, miR-378, miR-422 and miR-479[14] with 3 miRNAs: miR-21, miR-29a and miR-135b overexpressed in adenomas versus normal colonic tissue[15]; and also in feces (miR-21 and miR-106a, miR-92a and miR-106a)[16]. Regarding stool, which offers a miRNA profile very similar to that of colonic tissue, Ahmed *et al.*[17] proposed a panel of 12 miRNAs (miR-7, miR-17, miR-20a, miR-21, miR-92a, miR-96, miR-106a, miR-134, miR-183, miR-196a, miR-199a-3p, miR-214) with elevated expression in CRC, especially in metastatic CRC, compared to adenomas. The same authors described 8 miRNAs (miR-9, miR-29b, miR-127-5p, miR-138, miR-143, miR-146a, miR-222, miR-938) showing lower expression in CRC patients.

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The miRNA profile detected in our study does not allow us to ensure any individual or collective (cluster) signature defining colorectal lesion, although at least two miRNAs (miR-141-3p and miR-30d-5p) are differentially expressed in the EVs of first-degree relatives of patients with CRC compared to the control group, with differences between the two pathological groups. Furthermore, miR-92a-3p is overexpressed in patients with adenomas, whereas miR-141-3p is down-expressed (it is associated with 81 diseases in humans including, secondarily, CRC)[18]. This data is in line with several international studies that define diagnostic miRNA profiles, especially for CRC, although they do not refer to lesions with malignant potential or to relatives of CRC patients[19-22]. Although our work does not allow us to draw generalizable conclusions, the low number of samples is quite common in the literature, so that a study with a larger number of samples would allow us to define miRNAs with increased expression in pre- or neoplastic colorectal lesions.

The expression profile of tetraspanins (CD9, CD63 and CD81) detected by CF, which allows rapid and efficient detection, in first-degree relatives of CRC patients is different from that of the control group. In our investigation we detected a lower level of CD9 in subjects with AF CRC compared to controls. CD9 tetraspanin is involved in cell adhesion and EV uptake to colorectal tumor cells[23]. These processes are negatively regulated by tetraspanin CD9 expression on EVs. Tetraspanins CD81 and CD63 are involved in CRC progression and, moreover, it appears that the expression of both correlates with tumor invasion and metastasis. In our study, both groups with HF CRC presented lower CD81 values compared to controls, subjects with HF CRC and adenomas presented higher CD63 levels than in controls and the group with HF CRC and no adenomas showed lower levels. A study with a larger number of samples could perhaps define a proper differential profile of tetraspanins in urinary EVs for the different stages of colorectal pathology.

Conclusions

The present investigation allows us to highlight several aspects: 1) The validity of urine as a suitable diagnostic method for the study of EVs and the miRNAs carried in them. 2) The analysis of tetraspanins, which, although they are proteins used in CF as membrane markers, could complement or substitute the determination of miRNAs; that is, it could be sufficient to analyze the tetraspanin profile instead of miRNAs, which requires more time, personnel, material and economic resources as it involves genomic technology instead of CF (more versatile, cheaper and faster). And 3) This proof of concept may open a door for the possible design of a panel of Taqman probes that, by qPCR, study the expression of miRNAs in each at-risk subject in search of a non-invasive preventive diagnosis of colorectal lesions.

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CLINICAL CASE

GASTRIC INVOLVEMENT BY MULTIPLE MYELOMA: REPORT OF A RARE CLINICAL CASE WITH AN ATYPICAL INITIAL EXTRAMEDULLARY MANIFESTATION.

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Abstract

Plasma cell neoplasms include solitary plasmacytoma and multiple myeloma (MM), the latter being characterized by clonal proliferation of plasma cells within the bone marrow, often accompanied by systemic organ damage. Although extramedullary involvement is relatively uncommon, its presence is associated with increased disease aggressiveness and a poorer prognosis.

We report the case of a 54-year-old woman with no significant medical history who presented with cervical neurological symptoms. Imaging studies revealed a gastric mass initially suggestive of a gastrointestinal stromal tumor (GIST). Histopathological examination demonstrated infiltration by clonal plasma cells with a double monoclonal component—IgA lambda and free lambda light chains—confirming the diagnosis of multiple myeloma with gastric involvement.

This case underscores the importance of considering multiple myeloma in the differential diagnosis of atypical gastric masses and highlights the need for a comprehensive diagnostic workup when extramedullary disease is suspected.

Keywords: stomach, submucosal lesion, myeloma.

Introduction

Plasma cell neoplasms are a group of entities characterized by clonal proliferation of plasma cells, typically with a monoclonal component. They can manifest as a single lesion (solitary plasmacytoma) or as a systemic disease with bone marrow infiltration and organ damage (multiple myeloma)[1]. Solitary plasmacytoma is usually located in the skeletal system, although it can also occur in other tissues, in which case it is called extramedullary plasmacytoma. In the latter case, it is usually located mainly in the head and neck region, the upper airway, or the gastrointestinal tract, although gastrointestinal involvement is very rare[2].

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Sánchez Sánchez MI, Cano De La Cruz JD, Diego Martínez R, Mongil Poce L, Jiménez Pérez M. Gastric involvement by multiple myeloma: report of a rare clinical case with an atypical initial extramedullary manifestation. RAPD 2025;48(4):134-137. DOI: 10.37352/2025484.2

Multiple myeloma (MM) is characterized by the proliferation of plasma cells in the bone marrow, causing extensive bone destruction with osteolytic lesions, osteopenia, and/or pathological fractures[1]. In up to 7% of MM cases, extramedullary plasmacytomas are observed at diagnosis, and up to an additional 6% will develop extramedullary plasmacytomas during the course of the disease. In this context, positron emission tomography (PET) and computed tomography (CT) are crucial for diagnosis[1].

Although in most cases multiple myeloma presents exclusively with intramedullary involvement, in a significant percentage of cases extramedullary involvement is observed in the form of plasmacytoma, which usually implies greater aggressiveness of the disease and a worse prognosis[1].

In cases of gastrointestinal involvement, the most common location is the small intestine, usually diagnosed during disease follow-up, and rarely as an initial manifestation[2,3]. Gastric invasion is a rare manifestation of MM, presenting with nonspecific symptoms such as asthenia, decreased appetite, vomiting, or gastric masses that may mimic other entities[3].

Histologically, gastric involvement by multiple myeloma can be evidenced by dense infiltrates of malignant plasma cells in the gastric mucosa, which can cause complications such as vitamin B12 deficiency due to the destruction of gastric parietal cells[4]. In addition, gastric amyloidosis secondary to MM can mimic gastric cancer, so it is essential to perform a thorough diagnostic evaluation, including Congo red staining to detect amyloid deposits[5].

Clinical case

A 54-year-old woman with no relevant medical history presents with neck pain that has been ongoing for two months, associated with paresthesia and numbness in the upper limbs. A cervical MRI was requested, which showed a C6 fracture with posterior wall retropulsion and a soft tissue mass with stenosis at C5-C6 and C6-C7. Subsequently, it was decided to perform surgery with corpectomy along with cervical fixation and removal of the mass.

An extension study was performed with thoracoabdominal and pelvic CT, as well as PET-CT, which showed a 13 cm mass in the gastric wall, suggestive of a gastrointestinal stromal tumor, with evidence of distant disease. Given these findings, a gastroscopy and linear echoendoscopy were performed, identifying a large subepithelial mass affecting the gastric body and antrum. On ultrasound, the mass was hypoechoic with heterogeneous content, approximately 86 x 63 mm in diameter, and appeared to depend on the fourth layer or muscularis propria, with suspicion of a gastrointestinal stromal tumor with malignant degeneration and signs compatible with peritoneal carcinomatosis. A biopsy of the mass was performed with a 22G SharkCore needle without complications.

The pathological study of the surgical specimen and the sample obtained by USE revealed infiltration by plasma cells (clonal by flow cytometry). In addition, the patient had a double monoclonal component of IgA lambda and free lambda light chain, and was finally diagnosed with multiple myeloma with gastric involvement.

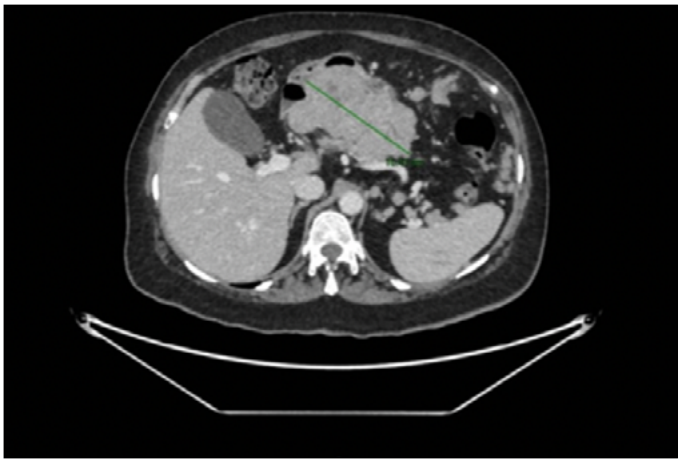

Figure 1. ***Key image from abdominal-pelvic CT scan. A 13 cm mass of probable submucosal origin is identified in the gastric wall with associated peritoneal carcinomatosis.***

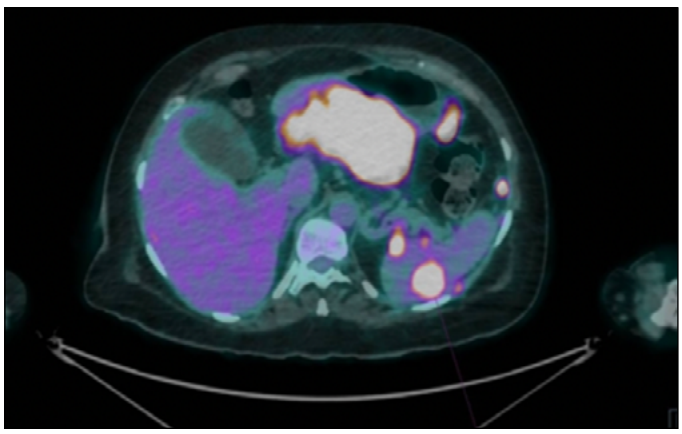

Figure 2. ***PET-CT scan at diagnosis. Image at gastric level with metabolic activity.***

The patient required prolonged hospitalization due to the slow progression of the disease due to refractoriness to treatment and complications arising from immunosuppression. Initially, first-line treatment was started with the D-VRd regimen, followed by second-line treatment with Kd-PACE, achieving a good response and allowing an autologous hematopoietic stem cell transplant to be performed. However,

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on day +100 post-transplant, radiological progression of the disease was identified, so it was decided to initiate third-line treatment with the Kpd regimen. Subsequently, a new endoscopic examination with gastroscopy was performed, in which no macroscopic lesions were observed. Currently, the patient remains hospitalized due to pancytopenia and febrile syndrome.

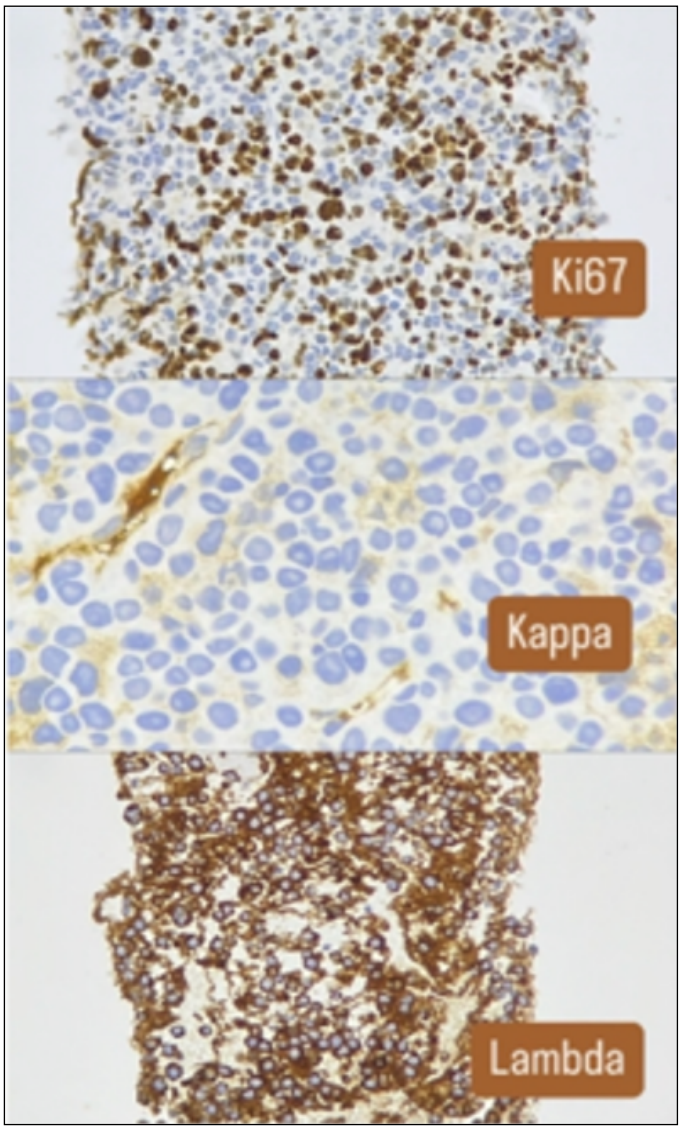

Figure 3. ***Anatomopathological sample obtained by linear echoendoscopy.***

Conclusion

Extramedullary plasmacytoma is a rare entity that can occur in isolation or in association with MM as an extramedullary manifestation of the disease. The prognosis for patients with extramedullary plasmacytomas is usually poor, with reduced survival, especially when associated with MM[1]. In the case of gastric involvement, it is more common to detect it in advanced stages of the disease[2,3].

The clinical symptoms of extramedullary plasmacytomas depend on their extent and may be due to three main mechanisms: direct invasion of an organ, mass effect, or myelomatous ascites[2]. In the case of gastric involvement, direct invasion usually causes symptoms such as nausea, vomiting, weight loss, upper gastrointestinal bleeding, or perforation[2,3].

In this case, the patient did not present with digestive symptoms, as the first manifestations were neurological, secondary to compression and involvement of the axial skeletal system, with gastric involvement detected in the extension study.

Endoscopically, gastric plasmacytoma lesions can present with various patterns, ranging from multiple mucosal ulcerations to single ulcerated masses, making it necessary to perform a differential diagnosis with other entities such as MALT lymphoma, gastric adenocarcinoma, GIST, neuroendocrine tumors (NET), and amyloidosis[3,4]. Biopsy for anatomopathological and immunohistochemical study is essential to confirm the diagnosis[2].

Treatment of solitary plasmacytomas includes surgical or endoscopic excision and, in some cases, radiotherapy[2]. When they occur in the context of MM, treatment follows the general principles of treatment for this neoplasm, with multidisciplinary management being essential. Systemic chemotherapy and immunomodulatory drugs are particularly important, and in some cases, autologous stem cell transplantation[1]. In cases of refractory gastrointestinal bleeding, radiotherapy, embolization of the bleeding vessel, or surgery may be used in cases of uncontrollable or recurrent bleeding, as well as in the presence of obstructive symptoms[2,5].

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CLINICAL CASE

DISIMPACTION OF FECALOMA WITH COCA-COLA® INJECTION

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Abstract

Fecalomas are hard stool formations, accumulated primarily in the distal colon and rectum, which can cause intestinal obstruction and serious complications. They commonly occur in the elderly or neuropsychiatric patients. Management is usually conservative, but when this is insufficient, surgical intervention may be required.

We present the case of a 75-year-old man with constipation of two weeks' duration. After an abdominal CT scan revealed a large fecaloma, conservative treatment was initiated, with no resolution of the condition. Subsequently, endoscopic treatment was performed with Coca-Cola® injections in several areas of the fecaloma, successfully breaking it up and thus normalizing the patient's bowel movement.

Keywords: fecaloma, constipation, impaction, Coca-Cola®.

Introduction

Fecal impactions are caused by a mass of hard stool that accumulates mainly in the distal colon and rectum. Factors that can contribute to their formation include chronic constipation, anorectal anatomical abnormalities, advanced age, and certain neuropsychiatric disorders. Management is usually conservative, involving oral laxatives, enemas, and sometimes even digital disimpaction. Complications are rare, but when they do occur, they may require surgical intervention[1].

Below, we present a case of a fecal impaction that did not resolve with conservative treatment and was successfully treated by endoscopic fragmentation with the help of Coca-Cola®, avoiding surgery for the patient.

Clinical case

A 75-year-old male with persistent constipation for two weeks, associated with general malaise and generalized

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abdominal discomfort. A plain abdominal X-ray was performed, which showed abundant fecal content in the colonic loops without ruling out loop suffering, so an abdominal CT scan was requested to complete the study. The CT scan showed a large fecaloma in the upper rectum measuring approximately 10 cm in diameter, as well as abundant fecal matter in the colonic frame, without complete obstruction of the colonic lumen (Figure 1). Despite intensive conservative treatment (oral laxatives, rectal enemas, Gastrografin®, etc.), the patient did not have a bowel movement. After consulting with general surgery and with surgery being the only treatment option, endoscopic disimpaction with Coca-Cola® was decided upon.

A colonoscopy was performed, revealing a large fecal impaction occupying almost the entire circumference of the colonic lumen, located about 10 cm from the external anal margin. Using a sclerotherapy needle, we proceeded to inject Coca-Cola® into several areas of the fecal impaction, softening the mass, and then, with the help of a wire loop, we achieved almost complete fragmentation of the impaction. Approximately 550 ml of Coca-Cola® was used in the procedure (Figures 2-4).

Following the endoscopic procedure, an enema was administered and oral laxatives were prescribed, with good subsequent progress. A rectoscopy was performed a few days later, which confirmed complete resolution of the fecal impaction and the absence of alterations in the rectal mucosa. The patient was subsequently discharged (Figure 5).

Discussion

Coca-Cola® is a well-known soft drink worldwide. It is used in the field of digestive endoscopy to treat certain gastrointestinal obstructive conditions such as gastric bezoars, thanks to its composition. The acidic nature of the beverage, due to phosphoric acid and carbonic acid, as well as the mucolytic effect of sodium bicarbonate and the carbon dioxide bubbles that penetrate through the microscopic pores on the surface of the bezoar, facilitates its fragmentation[2,3].

Several studies have documented the use of Coca-Cola® as an effective, safe, and low-cost alternative for dissolving gastric bezoars, constituting the first therapeutic option in many cases. However, evidence on its application in the treatment of fecal impaction is limited, and there are no standardized protocols that support its use in this context.

Fecal impactions are usually resolved with conservative measures such as laxatives or enemas. When these fail, surgery becomes the next therapeutic option[4,5,6].

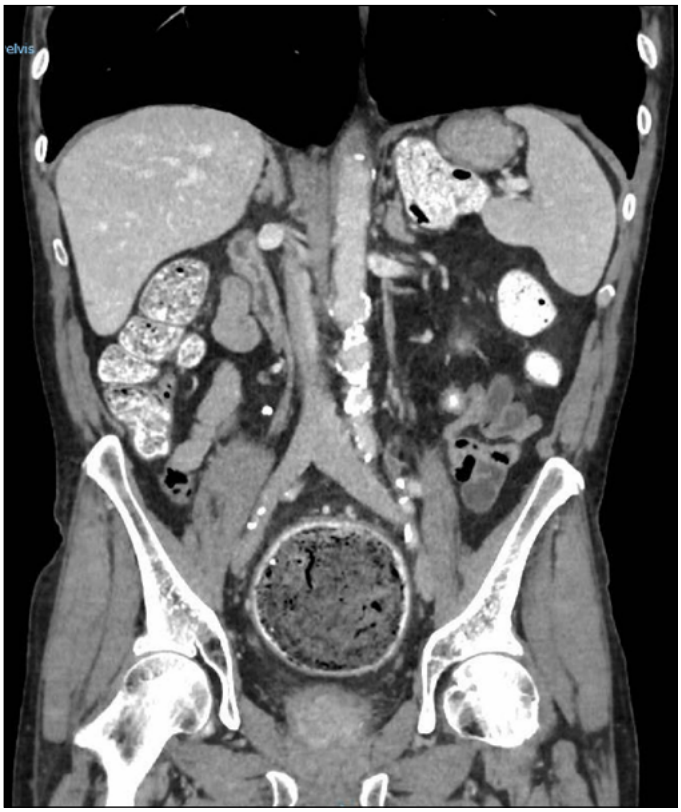

Figure 1. ***Coronal CT scan of the abdomen with contrast. A large fecal impaction measuring approximately 10 cm in diameter is visible in the upper rectum, along with abundant fecal debris in the colonic lumen.***

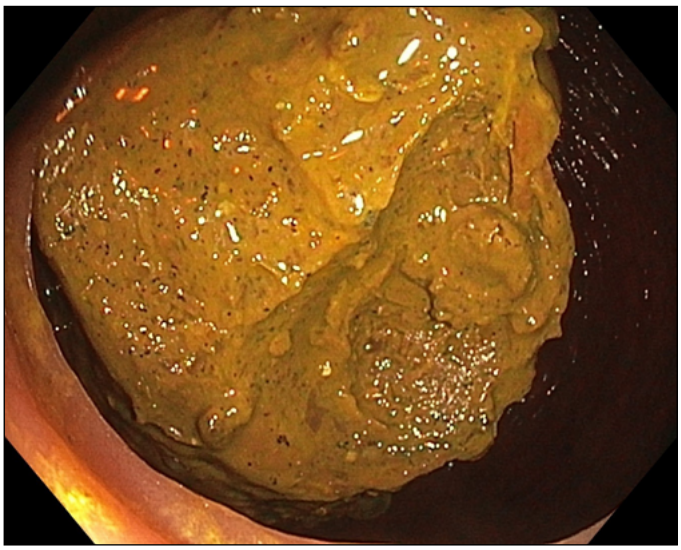

Figure 2. ***Endoscopic image of fecal mass approximately 10 cm from the external anal margin that does not completely obstruct the colonic lumen.***

In this clinical case, we present an endoscopic procedure in which the direct injection of Coca-Cola® into the fecal impaction allowed it to soften, facilitating its fragmentation with the wire loop. It is important to note that the beverage did not act alone as a resolving agent, but rather as an adjunct that enabled the success of the endoscopic procedure. We believe that, without this preliminary step, the exclusive use of the loop would not have been sufficient to resolve the problem.

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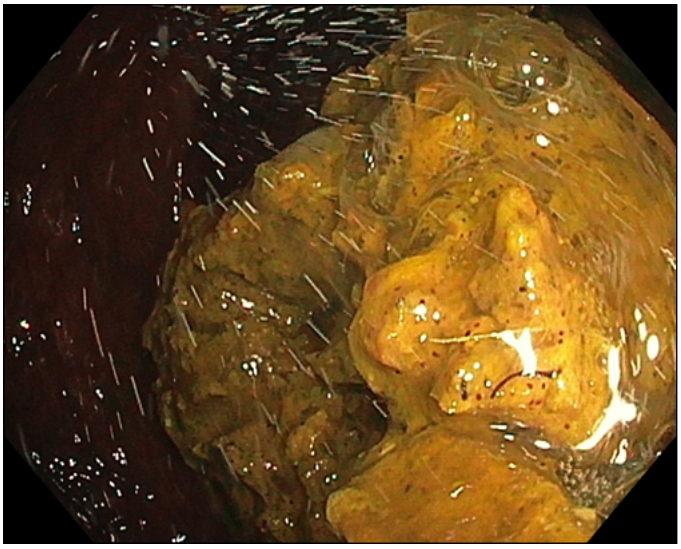

Figure 3. ***Partial fragmentation of the fecaloma. Small drops of Coca-Cola® are seen in the colonic lumen after injection into the walls of the stool mass.***

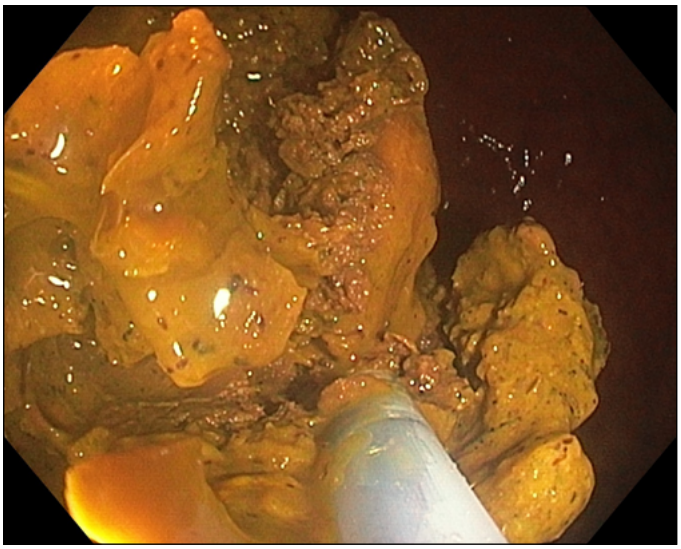

Figure 4. ***A decrease in the volume of the fecaloma is seen after several injections of Coca-Cola® through its walls.***

Although the strategy was effective and no lesions were observed in the rectal mucosa in the subsequent follow-up colonoscopy, we believe that it should be used with caution. Extrapolation from cases of gastric bezoars, where there is more evidence, should not be done automatically. This report represents a specific clinical experience and does not allow for general clinical recommendations to be made.

Although it appears to be a safe and effective procedure, a larger number of cases and systematic studies will be necessary to evaluate its safety, reproducibility, and efficacy in the treatment of refractory fecal impactions.

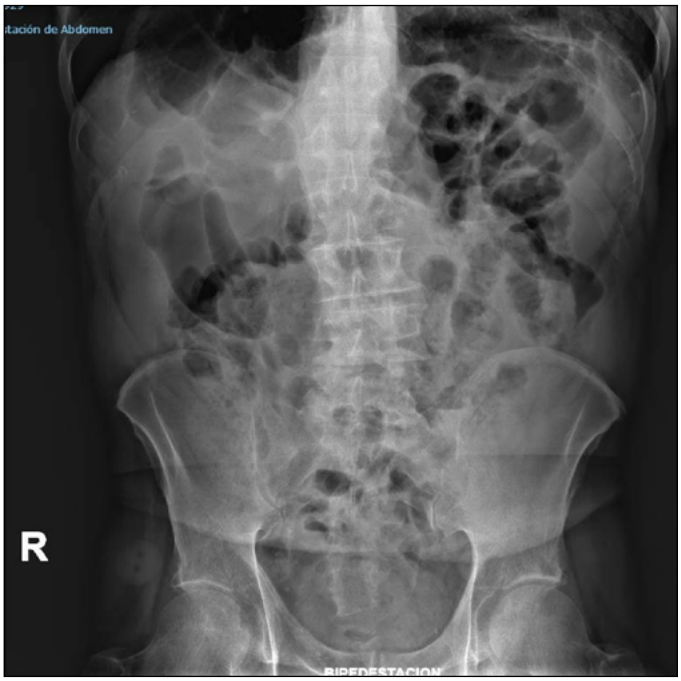

Figure 5. ***Plain abdominal X-ray in standing position. Performed 48 hours after the endoscopic procedure, showing the absence of rectal fecaloma.***

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CLINICAL CASE

SMALL INTESTINE HEMATOMA AFTER BICYCLE ACCIDENT

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Abstract

Blunt abdominal trauma can result in intestinal hematomas, most commonly affecting the duodenum, and presenting with a wide range of clinical manifestations—intestinal obstruction being the most frequent. This case report describes a jejunal hematoma following trauma, presenting atypically as gastrointestinal bleeding. The report also underscores the diagnostic value of video capsule endoscopy in identifying this type of pathology.

Keywords: jejunal hematoma, video capsule endoscopy, rectal bleeding.

Introduction

Intramural hematoma of the small intestine is a rare condition in abdominal trauma, occurring in less than 1% of closed trauma cases[1]. It is more common in children than in adults, and most reported cases occur in the duodenum, with jejunal hematoma being very rare[2,3].

We present a case of a male who, after a trauma while riding a bicycle, presented with overt rectal bleeding due to a jejunal hematoma, diagnosed by video capsule endoscopy.

Clinical case

A 50-year-old male with no relevant personal history, who is athletic, presents with overt rectal bleeding, hemodynamic instability, and anemia requiring transfusion. Upon completion of the medical history, he reports that 30 hours ago he accidentally fell from a bicycle from a height of two meters, causing blunt abdominal trauma. After hemodynamic stabilization, an oral endoscopy and colonoscopy were performed, revealing fresh blood in the colon and distal ileum without identifying any active bleeding points or potentially bleeding lesions, only isolated diverticula in the colon that did not show signs of bleeding. Initially, a diverticular origin of the bleeding was considered, but given that the patient continued

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Small intestine hematoma after bicycle accident.
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to have symptoms, and the colonoscopy had revealed abundant blood content in the ileum, it was necessary to rule out a small intestine origin, given the patient's history. It was therefore decided to complete the study with a Navicam SB video capsule endoscope with ProScan artificial intelligence (27 mm long and 11.8 mm in diameter). An extensive area of ecchymotic and markedly congested mucosa was identified in the mid-distal jejunum, with preserved villi and folds and blood remains at that level, suggestive of intestinal hematoma, with no other alterations at other levels, suggesting that this was the cause of the bleeding (Figures 1-3).

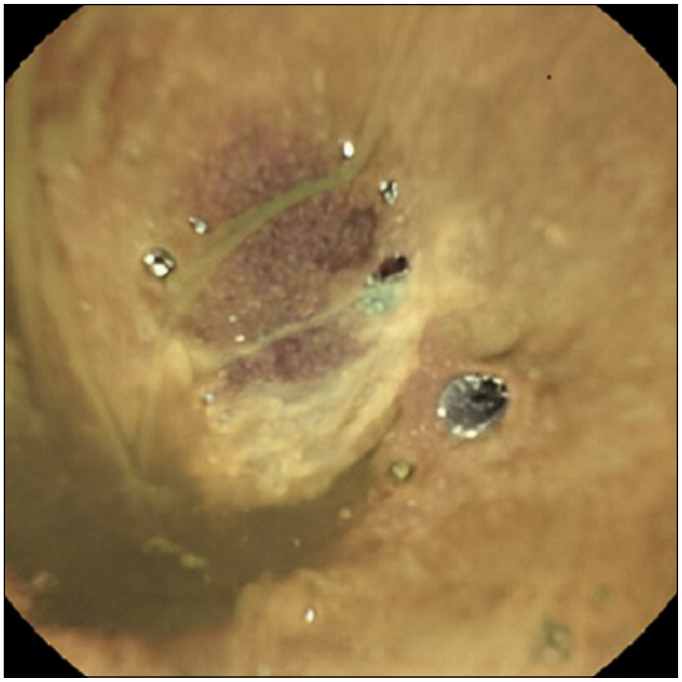

Figure 1. ***Endoscopic video capsule image showing markedly congestive ecchymotic mucosa without active bleeding at the mid-distal jejunum level.***

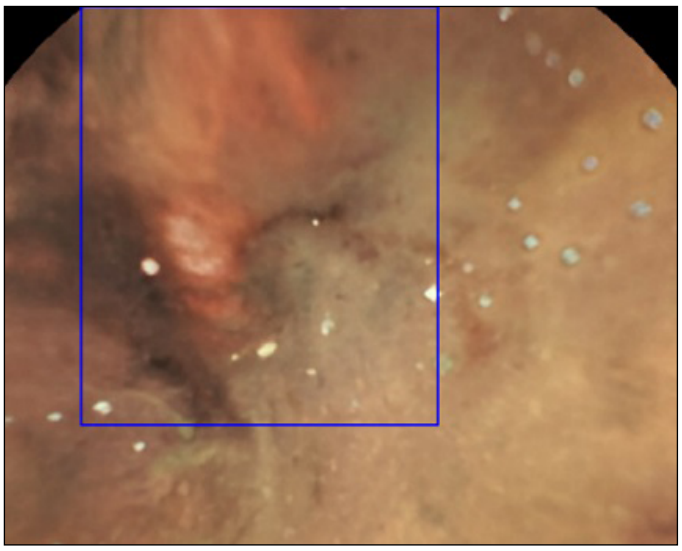

Figure 2. ***An area of equimotic intestinal mucosa, identified by artificial intelligence, suggestive of intramural hematoma.***

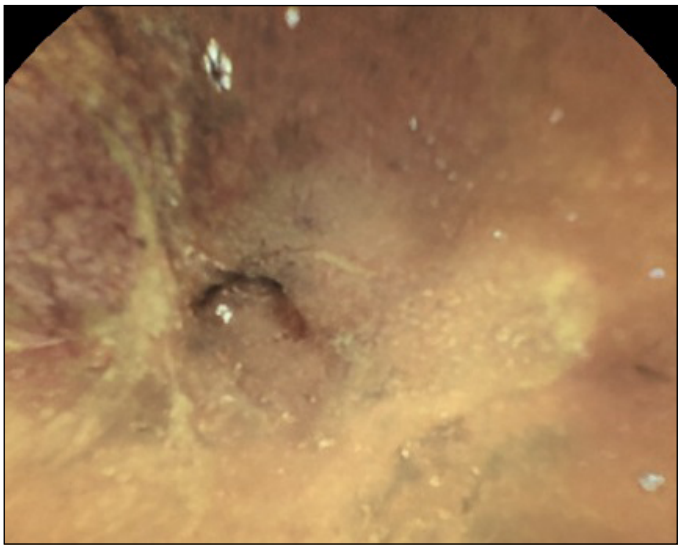

Figure 3. ***Image captured by endoscopic video capsule showing intramural hematoma at the mid-distal jejunum, approximately 2.7 cm in length.***

A conservative approach was adopted, with favorable clinical progress, no new episodes of rectal bleeding, and recovery of hemoglobin levels; therefore, the patient was eventually discharged.

Discussion

The clinical presentation of intestinal hematoma is highly variable and nonspecific, requiring a high degree of diagnostic suspicion. It can range from mild abdominal pain to intestinal obstruction, with rectal bleeding being a very rare and uncommon presentation[3]. The literature suggests that intramural intestinal hematomas are more common in people on anticoagulant therapy, with coagulation disorders, or who have undergone endoscopic procedures. Therefore, a thorough medical history, diagnostic suspicion, and appropriate complementary studies are very important[4].

Although there is no standardized protocol, a conservative approach is usually taken initially, as the outcome is usually favorable, with surgery being reserved for uncontrolled cases.

In this case, we highlight the importance of video capsule endoscopy as a diagnostic tool that allowed us to detect the lesion described, which was causing the clinical symptoms, thus avoiding unnecessary additional tests or new endoscopic studies.

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LIVER AMYLOIDOSIS AS AN EXCEPTIONAL CAUSE OF INTRAHEPATIC CHOLESTASIS

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Abstract

Hepatic amyloidosis is a rare manifestation of a systemic disease characterized by the extracellular deposition of insoluble proteins. It usually presents asymptomatically or with nonspecific symptoms, and cholestatic jaundice is an exceptional finding. We present a clinical case with this form of onset, in which imaging initially suggested a hepatic neoplasm, and the definitive diagnosis was established through liver biopsy.

Keywords: liver amyloidosis, cholestasis.

Introduction

Amyloidosis is a rare disease characterized by the extracellular deposition of insoluble proteins in the form of fibrils, which are resistant to proteolytic degradation. It can affect virtually any organ, causing progressive deterioration of its function. Hepatic involvement, although described, is rare and usually asymptomatic or with nonspecific symptoms. The presence of intrahepatic cholestasis and jaundice as an initial manifestation is exceptional. Below, we present a representative case of this unusual form of onset.

Clinical case

A 38-year-old patient with a history of obesity and type 2 diabetes mellitus presented to the emergency department with painless jaundice that had been present for one week. He reported progressive asthenia and weight loss of 5 kg in the last month. Laboratory tests showed total bilirubin of 8.1 mg/dL (direct 6.7 mg/dL), GGT 820 U/L, FA 1450 U/L, ALT 88 U/L, AST 72 U/L, and albumin 3.1 g/dL, with no significant elevation of tumor markers (CA 19.9, CEA, AFP). Abdominal ultrasound showed hepatomegaly, heterogeneous parenchyma with poorly defined hypoechoic areas, hyperechoic images suggestive of calcifications, and splenomegaly. The study was completed with computed tomography (CT), which showed multiple patchy hypodense areas in virtually all hepatic

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segments, some confluent, dilation of the intrahepatic bile duct, decreased intrahepatic vascular caliber, subcentimeter retroperitoneal adenopathies, and free fluid in the pelvis, initially pointing to intraductal cholangiocarcinoma (Figure 1). Given these findings, a liver biopsy was performed, which showed amyloid deposits positive with Congo red staining, confirming the diagnosis of hepatic amyloidosis.

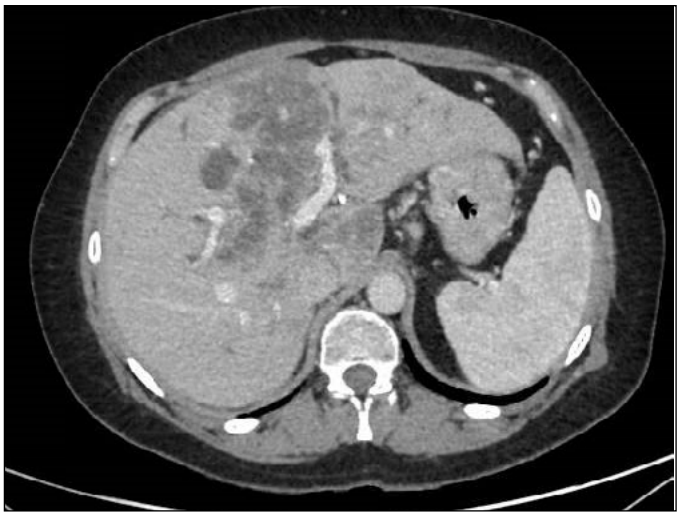

Figure 1. ***Heterogeneous liver with patchy hypodense areas associated with dilation of the intrahepatic bile duct and decreased caliber and attenuation of the different vascular branches.***

Discussion

Hepatic amyloidosis is a rare manifestation of systemic amyloid disease, usually associated with protein deposits in the hepatic sinusoids, portal space, or bile ducts. It usually presents asymptomatically or with vague symptoms such as asthenia, mild elevation of liver enzymes, or, less frequently, jaundice and intrahepatic cholestasis[1,2].

Imaging tests may show diffuse and heterogeneous liver involvement reflecting progressive liver damage due to amyloid infiltration, such as hypodense or hypocaptant areas that may mimic malignant lesions[3]. Liver biopsy with Congo red staining is the gold standard for diagnosis, allowing amyloid infiltration to be differentiated from other entities such as cholangiocarcinoma or lymphoma[4,5].

In this context, given the variety of clinical manifestations of hepatic amyloidosis, it is essential to maintain a high index of diagnostic suspicion, especially in patients with nonspecific symptoms and ultrasound findings suggestive of diffuse liver involvement.

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LETTER TO THE EDITOR

CHOLECYSTODUODENAL FISTULA: A RARE CAUSE OF INTESTINAL OBSTRUCTION, AN EXTRAORDINARY CAUSE OF HEMATEMESIS.

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Abstract

Cholecystoduodenal fistula is a rare complication of cholelithiasis. Symptoms are usually nonspecific, rarely presenting as biliary ileus and exceptionally as upper gastrointestinal bleeding.

We present a case of a 77-year-old patient with cholecystoduodenal fistula who presented with hematemesis diagnosed by endoscopy and computed tomography and later complicated by a biliary ileus in the form of Bouveret's syndrome. Finally, surgical management by enterolithotomy and open cholecystectomy was chosen; endoscopic hemostasis could not be achieved, but surgical treatment was successful.

Hemorrhage and biliary ileus associated with cholecystoduodenal fistulas usually require surgery because it is unlikely that treatment of the hemorrhage or removal of the lithiasis can be accomplished by conservative or endoscopic treatment. Cholecystoduodenal fistula should be considered as a differential diagnosis when a patient with a history of biliary disease presents with gastrointestinal bleeding.

Keywords: fistula , cholecystoduodenal , hematemesis, bouveret.

Introduction

Cholecystoenteric fistulas are a rare complication of gallstone disease. They are associated with ischemia and inflammation, which cause erosion and ultimately fistulization of the gallbladder wall. Among these, cholecystoduodenal fistulas are the most prevalent, with highly variable clinical presentation. In most cases, they are asymptomatic and are diagnosed incidentally during imaging tests or abdominal surgery; however, they can also occur, less frequently, in the form of recurrent cholangitis or pancreatitis, abscesses, intestinal obstruction due to biliary ileus, or, exceptionally, in the form of

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gastrointestinal bleeding[1]. With regard to biliary ileus, it should be noted that the terminal ileum is the most common site of impaction, but sometimes the stones become lodged in more proximal sections such as the jejunum or duodenum, causing gastric obstruction known as Bouveret's syndrome[2].

Clinical case

We present the case of a 70-year-old foreign patient with a personal history of chronic kidney disease on renal replacement therapy, symptomatic cholelithiasis, and abdominal surgery for which no reports are available. He came to the emergency room with epigastric pain and hematemesis with hemodynamic instability. Laboratory tests showed hemoglobin of 7.5 g/dL (previous 12.8 g/dL) along with worsening of baseline renal function with creatinine at 9.2 mg/dL. A gastroscopy was performed, which revealed upper gastrointestinal bleeding secondary to a duodenal ulcer of inflammatory origin, probably in the context of a foreign body, suggesting gallstones lodged at that level. An attempt was made to remove it, but this was unsuccessful, revealing an ulcer with an adherent clot and congestive edges without active bleeding in the underlying mucosa (Figure 1).

A CT scan of the abdomen was then performed due to suspicion of a cholecystoduodenal fistula. This imaging test reveals an 8 mm cholecystoduodenal fistula with air bubbles in the vesicular lumen and rarefaction of the perivesicular and periduodenal fat, without the duodenal lithiasis described in the endoscopy, together with an extraluminal contrast focus in the wall of the gallbladder, probably due to a small aneurysm (Figure 2).

The patient is discharged pending scheduled surgery, but returns a few weeks later with epigastric pain and vomiting with hemodynamic instability. A new abdominal CT scan is performed with findings that are compatible with Bouveret's syndrome secondary to cholelithiasis in the first portion of the duodenum due to a known cholecystoduodenal fistula that had increased significantly in size. He also presented with dilation of the gastric chamber and the first and second portions of the duodenum, which were occupied by hyperdense material suggestive of blood, with an active bleeding point observed on the wall of the gallbladder (Figures 3 and 4).

Based on the above, the patient underwent open cholecystectomy, with hemostasis of the aneurysmal bleeding and removal of the duodenal gallstones. He was subsequently discharged after treatment with empirical antibiotic therapy and good progress.

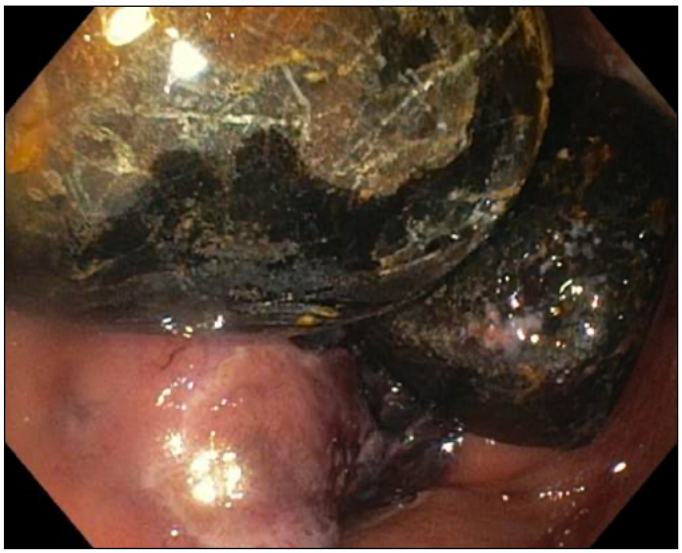

Figure 1. ***Upper gastrointestinal endoscopy image showing gallstones lodged in the duodenum, revealing an ulcer with an adherent clot and congestive edges in the underlying mucosa.***

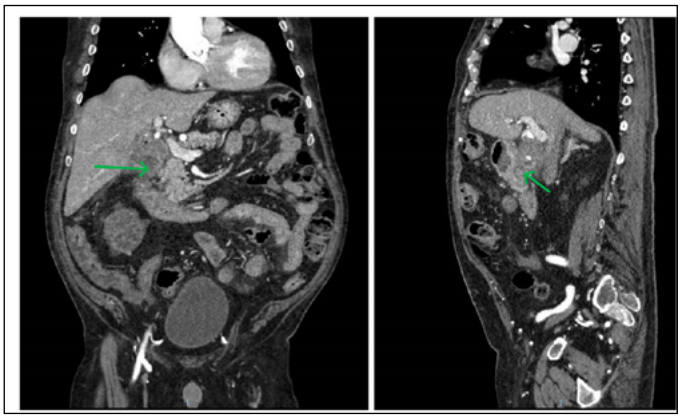

Figure 2. ***Coronal and sagittal CT scans of the abdomen with initial contrast, showing an 8 mm cholecystoduodenal fistula with rarefaction of fat adjacent to the gallbladder and duodenum.***

Discussion

The clinical presentation of a cholecystoduodenal fistula in the form of upper gastrointestinal bleeding, although well described in the literature, is a very rare finding. Invasion of the cystic artery by a duodenal ulcer can cause massive bleeding, and a gallstone can cause erosion of the same artery, thus causing bleeding. In the review conducted by Park JM *et al.*, endoscopic hemostasis was attempted in up to 4 of the cases described, but surgery was ultimately necessary in all of them[3,4]. In summary, gastrointestinal bleeding caused by a cholecystoduodenal fistula usually requires surgery, as it is unlikely that the bleeding will resolve with conservative treatment or endoscopic hemostasis.

On the other hand, the fact that this same patient subsequently developed Bouveret's syndrome is unusual. As in our case, the definitive treatment for this complication is

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surgical, although there is no consensus on the appropriate surgical procedure. The most commonly used procedure is enterolithotomy with open cholecystectomy, given the chronic inflammation and anatomical distortion[1,2].

In conclusion, cholecystoduodenal fistulas should be considered in the differential diagnosis when a patient with a history of biliary disease presents with gastrointestinal bleeding.

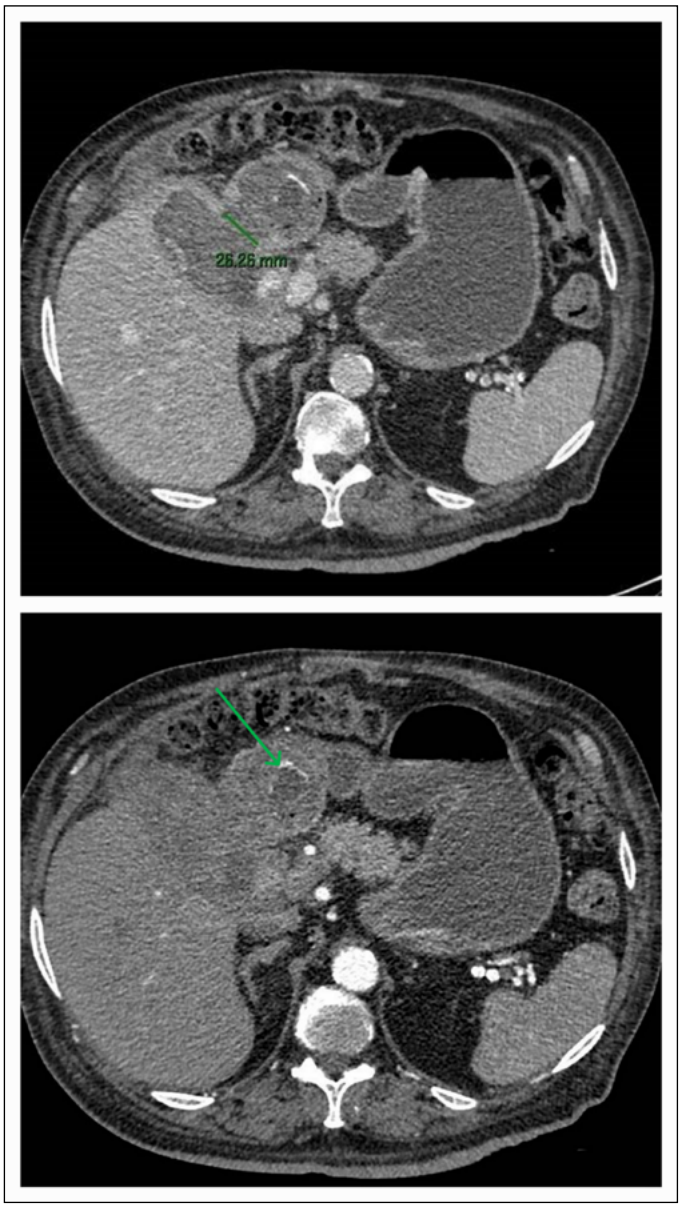

Figure 4. ***Bouveret's syndrome secondary to passage of a 2.6 cm cholelithiasis to the first portion of the duodenum through a cholecystoduodenal fistula that has increased significantly in size. Occupation of the gastric chamber and first and second portions of the duodenum by blood material.***

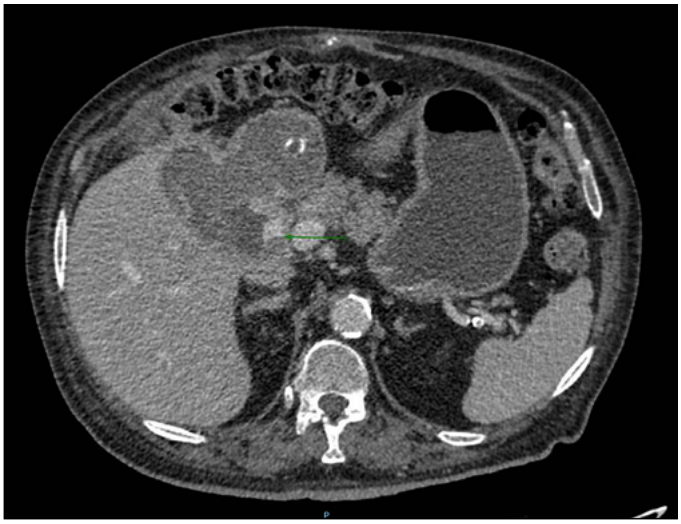

Figure 4. ***Active bleeding point in the gallbladder wall near the infundibulum, in the area where a small pseudoaneurysm was described in the previous CT scan.***

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