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Review Article

Original Articles

Clinical Reasoning

Case Report

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EDITORIAL POLICY

Scope and Mission

Albanian Journal of Medical and Health Sciences *(AJMHS)* is a quarterly published, peer-reviewed open-access international journal. The journal is the official scientific publication of the University of Medicine, Tirana, Albania. The language of the journal is English.

AJMHS was founded in 1961 as "*Buletini i Shkencave Mjekësore i Fakultetit të Mjekësisë, Universiteti i Tiranës" (Bulletin of Medical Sciences-Faculty of Medicine, University of Tirana*). In 2012 the journal was published in English for the first time as "*Bulletin of Medical Sciences*". The journal's name changed to "*Albanian Journal of Medical and Health Sciences*" in 2014.

AJMHS publishes scientific articles in basic, translational, clinical and health care research, conducted in all fields of medicine and health care, as well as interesting case reports and clinical images, invited reviews, invited medical education papers, editorials, opinions and viewpoints, comments and letters to the Editor. The structure of each edition of the publication comprises section categories determined by the Editor and reflects the views of the Editorial Board.

AJMHS encourages academicians, researchers and specialists of different medical and health care fields from all over the world to publish their valuable research in all branches of medicine and health care.

The journal's aim is to publish original articles with high scientific and ethical quality.

The Editorial Board of the *AJMHS* and the Publisher adhere 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), the US Office of Research Integrity (ORI), the European Association of Science Editors (EASE), and the International Society of Managing and Technical Editors (ISMTE).

AJMHS permits and encourages authors to post items approved for publication from the journal on personal websites or institutional repositories both prior to and after publication, while providing bibliographic details of the publication in *AJMHS*. All articles are also available in PDF format on our website http://ajmhs.umed.edu.al and can be downloaded free of charge.

The *AJMHS*'s mission is to distribute and expand worldwide good quality research, focused primarily on the medical and health care problems of the South-East European and Mediterranean countries.

AJMHS is open to publication for all the authors that comply with the scientific and ethical requirements of

the journal. All manuscripts submitted for publication are strictly internally and externally peer reviewed for their originality, methodology, scientific relevance, quality, ethical nature and suitability for the journal. A similarity check is performed on all manuscripts submitted. All the articles published at *AJMHS* will be fully accessed online. No submission or publishing fee is requested.

Ethics

AJMHS is committed to the highest standards of research and publication ethics. All submitted manuscripts are screened for plagiarism in order to detect instances of overlapping and similar text. The editors will act in accordance to the relevant international rules of publication and research ethics (COPE guidelines, WAME resources, WMA policies and ORI) if any ethical misconduct is suspected.

The journal recommends an approval of the 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)". This approval is required for all experimental, clinical and drug trial studies. For articles concerning experimental research on humans, a statement should be included that 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. More details on the ethical principles of the journal may be found at the "Ethical Guidelines" and the "Instructions to Reviewers" pages. All reference for the ethical issues must be mentioned at the method section of the article.

Conflict of interest policy

The AJMHS's editorial review process is in accordance with the Good Editorial Practice set by international editorial organizations (WAME, COPE). WAME indicates that "conflict of interest exists when an author, reviewer, or editor in the publication process (submission of manuscripts, peer review, editorial decisions and communication between authors, reviewers and editors) has a competing interest that could unduly influence his or her responsibilities (academic honesty, unbiased conduct and reporting of research and integrity of decisions or judgments) in the publication process".

The AJMHS requires that each author, reviewer, and editor must disclose to the editor-in-chief any conflict of interest related to family, personal, financial, political or religious issues as well as any competing interest outlined above at the WAME's definition. Whether or not a conflict of interest and financial support exist, they must be declared at the Conflict of Interest Statement (signed and approved from all the authors) as well as at the end of the manuscripts (Conflict of Interest Statement, before the Reference Section). If a reviewer or an editor has a conflict of interest and/or believes that it is not appropriate to be a reviewer, or an editor for a given manuscript, the reviewer or the editor should resign from the assignment. The AJMHS editorial board members may also submit their own manuscripts to the journal. However, they cannot take part at any stage on the editorial decision of their manuscripts. They will be treated like any other author and if any, final acceptance of such manuscripts can only be made by the positive recommendation of at least two external reviewers.

Authors should not contact any of the editorial executive or scientific board members during the review process. All necessary information regarding the process of a manuscript will be regularly provided from the editorial office via the official e-mail addresses. The names of the handling editor and the reviewers are not disclosed to the author(s). Due to the AJMHS's double-blinded review principles, the names of authors and reviewers are not known to each other. Please refer to the "conflict of interest statement and copyright form" section below for the conflict of interest declaration for authors. For a conflict of interest statement for reviewers, please refer to the "Instructions to Reviewers" page.

INSTRUCTIONS FOR AUTHORS

AJMHS is based on independent and unbiased double-blind and peer-reviewing principles. Only unpublished papers that are not under review for publication elsewhere can be submitted. The authors are responsible for the scientific content and the ethical compliance of the material to be published. *AJMHS* reserves the right to request any research materials on which the paper is based. It is highly recommended that all manuscript must be checked from a native English speaker with experience in Scientific English writing. The executive editorial board is committed to a rapid publishing process. The

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authors will be kept informed about all the stages of the reviewing process.

Manuscript formatting

Manuscript format must follow the guidelines described below that are in accordance with the ICMJE (Recommendations for the Conduct, Reporting, Editing and Publication of Scholarly Work in Medical Journals - updated in August 2013 - http://www.icmje.org/icmje-recommendations.pdf).
The manuscript must be submitted to the following address: ajmhs.submission@gmail.com
Papers that do not comply with the format of the Journal and submission requirements will be returned to the author for correction without further review.

General Format

The manuscript should be typed in a Microsoft Word™ file, single-column format, double-spaced with 2.5 cm margins on each side, and 11-point type in Times New Roman font.

All abbreviations must be defined the first time they are used and should be displayed in parentheses after the definition. Abbreviations should be limited to those defined in the AMA Manual of Style, current edition. Authors should avoid abbreviations in the title and abstract and limit their use in the main text.

Decimal points should be used in decimals throughout the manuscript. Measurements should be reported using the metric system according to the International System of Units (SI). Consult the SI Unit Conversion Guide (New England Journal of Medicine Books, 1992). An extensive list of conversion factors can be found at:
http://www.unc.edu/~rowlett/units. For more details, see:

http://www.amamanualofstyle.com/oso/public/jama/si_conversion_table.html.

When a drug, product, hardware, or software is mentioned within the main text product information, it should include the name of the product, the producer of the product and the city or the country of the company .It should be provided in parenthesis in the following example format: "Examination BIO-AUTO analyzer (Beckman-Coulter, New Jersey, NJ, USA)".

Article Type
Identification of the article type is the first step of manuscript preparation and submission. The article type dictates the rules that should be followed, including formatting and word limits of the manuscript. The main categories of article types are outlined below:

Original Article: Original contributions are manuscripts containing substantial novel research. These articles can include randomized controlled trials, observational (cohort, case-control or cross-sectional) studies, diagnostic accuracy studies, systematic reviews and meta-analyses, non-randomized behavioral and public health intervention trials, experimental animal trials, or any other clinical or experimental studies. Abstracts must begin on a separate page and should not exceed 400 words. Abstracts should be structured with the following subheadings: Background, Aims, Study Design (case control study, cross-sectional study, cohort study, randomized controlled trial, diagnostic accuracy study, meta-analysis and systemic review, animal and in vitro experimentation, non-randomized study in behavioral sciences and public health, etc.), Methods, Results and Conclusion. The main text should be structured with the following subheadings: Introduction, Material and Methods, Results, Discussion, Conclusions, Acknowledgments, Conflict of Interest statement, Authorship contribution, References, Tables, and Figure Legends. The main text should not exceed 3500 words, excluding the abstract, references, tables, and figure legends. There should be a maximum of 40 references.

Short Report: Short reports or short communications are short versions of research, applications or work in progress limited to 1500 words. These articles can include clinical or laboratory work, collected case reports of scientific significance etc. Abstracts must begin on a separate page and should not exceed 250 words. Abstracts should be structured with the following subheadings: Background, Aims, Study, Methods, Results and Conclusion. The main text should be structured with the following subheadings: Introduction, Material and Methods, Results, Discussion, Conclusions, Acknowledgments, References, Tables, and Figure Legends. The main text should not exceed 1500 words, excluding the abstract, references, tables, and figure legends. There should be a maximum of 4 tables and/or figures and 15 references.

Invited Review or Medical education articles: Invited review and Medical education articles are comprehensive analyses of specific topics in medicine, which are written upon invitation due to the extensive experience and publications of authors

on the review subjects. They can also be articles focused on clinical teaching and guidelines. All invited review articles will also undergo peer reviewing prior to acceptance. Review articles must not exceed 5000 words for the main text (excluding references, tables, and figure legends) and 400 words for the unstructured abstract. A review article can be signed by no more than 5 authors and can have no more than 60 references.

Case Report: Interesting cases demonstrating new findings can be reported. Cases should be unique, representing a diagnostic or therapeutic challenge and having a learning point for the readers. Abstracts of case reports should mainly include information about the case and should be limited to a maximum of 250 words. The abstract must begin on a separate page and should be structured with the following subheadings: Background, Case Report and Conclusion. The main text of case reports should be structured with the following subheadings: Introduction, Case Report, Discussion, Acknowledgments and References. Case reports must not exceed 1200 words (excluding references, tables, and figure legends). Case reports can be signed by no more than 5 authors and can have no more than 10 references and 3 figures or tables. It is highly recommended that the Case reporting must follow the CARE (Case Report) guidelines.

Clinical Reasoning: Clinical reasoning represents a rational thinking through the various aspects of patient care to better define the medical strategy regarding the diagnosis and/or treatment of a clinical problem in a specific patient. Conducting a physical exam, taking a medical history, ordering complementary exams and describing safe and effective treatment are necessary steps in gathering clinical data from a patient before engaging in the process of clinical reasoning. The latter represents a critical thinking process about all the important clinical information using personal skills and abilities often achieved from the experience. This article type is intended to help clinicians think differentially and take the next step which determines the best course of action to take based on what is known or what can reasonably be hypothesized from clinical data. The authors are encouraged to present clinical cases from their experience which has generated a real diagnostic dilemma. The first section, case presentation, should include the patient's complaints as well as historical and clinical data enough to present an initial differential diagnosis. The second section, complementary exams, is dedicated to pertinent and necessary complimentary examinations according to previous topographic and clinical differential diagnosis. In the third section, the authors should present all steps (surgery, biopsy, pathological exam) needed in defining the final diagnosis. A supplementary section should include an overview of the final diagnosis. The maximum lengths of the text and the references should not exceed 2000 words and 20 references, respectively. No abstract is required.

Clinical Image: The journal publishes original, interesting, and high quality clinical images having a brief explanation (maximum 500 words excluding references but including figure legends) and of educational significance. The figure legend should contain no more than 100 words. It can be signed by no more than 5 authors and can have no more than 5 references and 1 figure or table. Any information that might identify the patient or hospital, including the date, should be removed from the image. An abstract is not required with this type of manuscripts. The main text of clinical images should be structured with the following subheadings: Case, and References.

Letter to the Editor: Letters in reference to a journal article must not exceed 500 words (excluding references). Letters not related to a journal article must also not exceed 500 words (excluding references). An abstract is not required with this type of manuscripts. A letter can be signed by no more than 4 authors and can have no more than 5 references and 1 figure or table.

Other: Editorials, reviewer commentaries, book reviews, reports on publication and research ethics, Opinions and View-Points are requested by the Editorial Board.

A summary of the article type's characteristics is given in the table below.

Article Type	Word Limit	Abstract word Limit	Reference Limit	Author Limit	Tables/figures Limit
Original Article	3500[1]	400[4]	40	None	6
Invited Review	5000[1]	400	60	5	6
Case Report	1200[1]	250[5]	10	5	3
Clinical Image	500[2]	N/A	5	5	1
Letter to the editor	500[3]	N/A	5	4	1
Clinical reasoning	2000[3]	N/A	20	5	3
Short report	1500	250[4]	15	10	4

AJMHS 2016; Vol. 47 (2/3): vii-xvii © 2016

1 This should not include the abstract, references, tables or figure legends.
2 This should include the figure legends.
3 This should not include the references.
4 Should be structured with the following subheadings: Background, Aims, Study Design, Methods, Results, and Conclusion.
5 Should be structured with the following subheadings: Background, Case Report and Conclusion.

Preparation and submission of a manuscript

All manuscripts should be submitted via email to the following address:
ajmhs.submission@gmail.com
The submission should be divided into SEPARATE files in the following order:

1. Cover Letter (separate file).
2. Authorship Contributions, Copyright Transfer and Conflict of Interest Statement Form (separate signed file).
3. Manuscript (Title page, Abstract page, main text, references, tables, and figure legends).
4. Figures (if applicable).

1 - Cover Letter

The cover letter, addressed to the Editor In Chief from the corresponding author, should include: the article title and type of article he/she is submitting (for example: original article, case report, review article or clinical image). The corresponding author should briefly summarize why their work is a valuable addition to the scientific literature. Furthermore, there should be a statement that the manuscript has not already been published, accepted or under simultaneous review for publication elsewhere. AJMHS does not accept multiple submission and duplicate submission. For manuscripts that have been presented orally or as a poster, this must be stated on the title page with the date and the place of the presentation. An example of a cover letter can be found on the journal's webpage (AJMHS Cover Letter).

2 - Authorship Contributions, Copyright Transfer and Conflict of Interest Statement Form

This is a statement of scientific contributions and responsibilities of all authors. The form is available for download at the the journal's webpage. The ICMJE recommends that authorship has to be based on the following 4 criteria:

1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. 2. Drafting the work or revising it critically for important intellectual content. 3. Final approval of the version to be published. 4. 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.

A contributor should meet all four criteria to be identified as an author. If a contributor does not meet all four criteria, he/she should be acknowledged in the acknowledgements section of the manuscript. All authors must sign the corresponding declaration.

For more details please refer to the ICMJE's definition of the role of authors and contributors at: http://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html.

AJMHS recommends that the author ranking in the authorship list has to follow the importance of the contribution of the individual co-authors in the study, with the exception of the last author who is generally the author group coordinator or leader and whose contribution is comparable with the first author. The authors must state in the section dedicated to the Author Contribution Form and in the main text (before the Reference section), if they have agreed for another ranking order (for example: authors A.B and C.D. have an equal contribution to this study, etc). The specific contribution of each author must be stated at the end of the manuscript, before the references.

All contributing authors must sign the **Authorship Contributions, Copyright Transfer and Conflict of Interest Statement Form** and submit it through the submission system during submission. Please see Authorship Contributions**,** Conflict of Interest Statement and Copyright form for detailed information regarding "Acknowledgement of Authorship, Exclusive Publication Statement, Conflict of Interest Statement, and Transfer of Copyright Agreement".

Please refer to "conflict of interest policy" for more information.

3 - Manuscript must contain:

Title Page (separate page)

This should include:

a - The complete manuscript title (no more than 150 characters).

b - The running head (no more than 50 characters).

c - Word counts for the abstract and text (the text word count does not include references, tables, and figure legends).

d - The number of references and the number of figures and/or tables.
e - All authors' full names.
f - Detailed affiliations and e-mail addresses (all authors should meet the ICMJE's requirements for authorship – see details at "author contribution form").
g - The name, address, telephone and fax numbers and email address of the corresponding author.
h - Key-words: (3 to 6 key-words) from the list provided in Index Medicus under "Medical Subject Heading (MeSH)".
e - Information about where and when the study has previously been presented.

Abstract Page (separate page)
Original articles, invited review articles and case reports should include an abstract in a separate page. Abstracts for original articles and short reports should be structured with the following subheadings: Background, Aims, Study design, Methods, Results, and Conclusion. Abstracts for case reports should be structured with the following subheadings: Background, Case Report, and Conclusion. Abstracts for review articles should not be structured. Clinical images, clinical reasoning, Editorials, Letters to the Editor, and Commentaries or Opinions/Viewpoints should not contain an abstract.

Main document
The main document should include the main text, acknowledgements, conflict of interest disclosure, authorship contribution description, references, tables, and figure legends, in that order.

Main text
The main text should be structured according to the article type, as described in the Article Type section above.

Acknowledgements
All contributors who do not meet the criteria for authorship (ICMJE: authorship and contributorship: http://www.icmje.org/ethical_1author.html) should be mentioned in this subheading.
Conflict of interest disclosure: The authors should disclose any potential conflict of interest.
Statement about specific author contribution at the study (including concept, design, supervision, resource, materials, data collection and/or processing, analysis and/or interpretation, literature search, writing and critical reviewing). For example: A.B (concept, design, data collection etc); B.C. (data collection, analysis, writing, reviewing etc). They should comply with ICMJE recommendations that authorship be based on the following 4 criteria: 1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work. 2. Drafting the work or revising it critically for important intellectual content. 3. Final approval of the version to be published. 4. 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.
A contributor should meet all four criteria to be identified as an author. If a contributor does not meet all four criteria he/she should be acknowledged in the acknowledgements section of the manuscript.

References
Authors are encouraged to cite primary literature rather than review articles in order to give credit to those who have performed the original work. Reference listings must be in accordance with ICMJE standards and numbered consecutively at the end of the manuscript in the order in which they are mentioned in the text. While citing publications, preference should be given to the latest, most up to date publications. Full papers must be clearly differentiated from abstracts presented in scientific meetings and published as supplements in scientific journals (see below: Abstract example). If an ahead of print publication is being cited the 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 Index Medicus/ Medline/Pub Med (for journal abbreviations consult the List of Journals indexed for MEDLINE, published annually by NLM). When there are 6 or less authors, all authors should be listed. If there are 7 or more authors, the first 6 authors should be listed followed by "et al". In the main text of the manuscript, references should be cited using Arabic numbers in parentheses. The reference styles for different types of publications are presented in the following examples:
Journal article: Korini G, Kocova O, Abani L, Vikani E, Vini T. Polymorphisms of cytochrome P464 genes in three ethnic groups from Albania. Albanian J Med Health Sci 2012;29:252-60.
Book: Benon M. Ocular manipulation. 1st ed. Stuttgart-New York: Georg Thieme Verlag; 2009.
Book chapter: Tos M, Stangerup SE. The relationship between secretory diarrhea and nutrition. In: Mos F, Thompton J, Peitersen E, editors. Nutrition and medical treatment. Amsterdam: Kugler & Ghedini; 1989:325-30.
Abstract: Gurakar A, Elsahwi K, Akdogan M, Wright H, Nour, B, Sebastian T, et al. Asplenia and

primary sclerosing cholangitis (PSC): A mere coincidence? Hepatology 2002;36:673a (abstract).
Article 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: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2626828/pdf/8903148.pdf.
For other reference style, please refer to "ICMJE Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Sample References".

Tables
Tables should be presented within the main document and after the reference list. All tables 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. A descriptive title should be provided for all tables and the titles should be placed above the tables. Abbreviations used in the tables should be defined below the tables (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 arranged clearly to provide an easy reading.

Figures and Figure Legends
Figures, graphics and photographs should be submitted as separate files (in TIFF or JPEG format). They should not be embedded in a Word document. When there are figure subunits, the subunits should be labeled in small letters (a, b, c, etc.). Thick and thin arrows, arrowheads, stars, asterisks and similar marks can be used on the images to support figure legends. Like the rest of the submission, the figures should be blind too. Any information within the images that may indicate 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. When there are figure subunits, the figure legends should be structured in the following format.
Example: Figure 1. a-c. Primary culture of choroid plexuses on day 2 after seeding of dissociated cells (×400). Nesting staining in green (a). GFAP staining in red (b). Nuclear labeling in blue and merged images (c).

Checklist
Before submission, the corresponding author should ensure that all files mentioned below meet the journal requirements:
1. A cover letter containing
- The article title and type
- A brief statement describing the novelty and importance of the work
- A statement declaring the absence or presence of a conflict of interest
- A statement that the manuscript has not been previously published or accepted for publication and is not submitted or under simultaneous review for publication elsewhere.

2. The author contribution, Transfer of Copyright Agreement and Conflict of Interest Statement Form (all in a separate signed file) is included and signed from all the authors.
3. A title page including
- Title (less than 150 characters), running title (less than 50 characters)
- Authors' affiliations and e-mail addresses, including the name of the corresponding author
- Key words: 3 to 6 key-words
- Word count for the abstract and main text
- A statement of the date and place of the meeting where the manuscript was presented orally or as a poster, if occurred.

4. Structured Abstract (on a separate page-see above)

5. Structured Main text (see above)
- Ethical approval and/or informed consent has to be mentioned in the text (Methods)
- References are in the correct format and cited sequentially in the text
- All Tables and Figures have been included and appear correctly

6. Permission for reprinted figures, tables, materials or photographs has been obtained (if available)

REVIEWING PROCESS

Revisions
When submitting a revised version of a paper, the author must submit a detailed "Response to reviewers" reporting in great detail how each issue raised by the reviewers was covered.
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 will be automatically cancelled. If the submitting author(s) believe that additional time is required, they should

request a 2-week-extension before the initial 30 day period is over.

Change of authorship and withdrawal request

Any request to change the author list after submission, such as a change in the order of the authors or the deletion or addition of author names, is subject to the Editorial Board's approval. In order to obtain this approval, please include in a letter to the editor the following information: 1 - The reason for the change of authorship. 2 - Signatures of all authors (including the new and/or removed author).

Please note, if you are adding or removing author/authors, a new copyright transfer form signed by all authors should also be sent to the editorial office after the Editorial Board approves the change of authorship.

All withdrawal requests at any stage after submission are evaluated by the Editorial Board. The *Albanian Journal of Medical and Health Sciences* has the right to not accept a withdrawal request. The authors should explain their reason to withdraw the paper by a detailed letter. If the reason of withdrawal is not justified by the Editorial Board, the authors of the paper can be banned for up to 1 (one) year from submitting a new paper to the Journal.

Ethical guidelines

AJMHS is committed to the highest standards of research and publication ethics. If ethical misconduct is suspected, the Editorial Board will act in accordance with the relevant international rules of publication ethics (i.e. COPE guidelines, WAME resources, WMA policies and ORI).

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, 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 experimental and clinical and drug trial studies. 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. Informed consent must also be obtained for case reports. All recognizable photographs of a patient must be accompanied by written permission from the patient for reproduction. Procedures that were performed to eliminate any pain, harm and distress in subjects/animals should clearly be stated. The authors should clearly state their compliance with internationally accepted guidelines and the guidelines issued by the relevant authority of their country. The journal requests a copy of the Ethics Committee Approval received from the relevant authority.

All authors should meet the ICMJE's authorship criteria outlined at the "authorship contribution form" section. *AJMHS* does not accept gift, guest, or ghost authorship, and will act according to the COPE guidelines and flowcharts when faced with cases of suspected misconduct.

Plagiarism Detection

AJMHS does not allow any form of plagiarism. In accordance with our journal policy, submitted manuscripts are screened with plagiarism software to detect instances of overlapping and similar text at least two times (during the evaluation process and after acceptance).

Appeals and complaints

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 the Editor in Chief. An executive Editor or the Ethical Committee of the University 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 ajmhs.editor@gmail.com.

Proofs and DOI number

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

Presubmission inquires and rapid evaluation requests

Presubmission inquires and rapid evaluation requests can be sent to the *AJMHS*.

Presubmission inquiries are usually sent by the authors to determine if a possible submission of their current work to *AJMHS* would receive a high enough priority for publication. These inquiries are handled by the Editor in Chief and the Editorial Board and a positive feedback from the Editor in Chief does not guarantee the publication of the work in question as all manuscripts submitted to *AJMHS* must be peer reviewed. Presubmission inquiries should be sent to ajmhs.editor@gmail.com via e-mail and should include a shorter version of the cover letter

AJMHS 2016; Vol. 47 (2/3): vii-xvii © 2016

accompanied by the title and the abstract of the manuscript.
Rapid evaluation requests are usually sent by the authors to state a particular importance of their current work which requires the manuscript to be evaluated as quickly as possible. These requests are handled by the Editor in Chief of the journal; should be sent to ajmhs.editor@gmail.com and should include a shorter version of the cover letter explaining the importance of the manuscript accompanied by the title and the abstract of the manuscript.
Non-scientific reasons, such as academic career needs, will not be considered and may result immediate reject of a manuscript. Authors are not allowed to contact reviewers for their manuscripts. This is an unacceptable behavior and may lead to the rejection of the manuscript and the author/s may be banned for further submissions to the journal. Any question related to the editorial process should be forwarded to the secretariat, managing editor or the editor-in-chief.

Documents to download

- Cover Letter
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Establishing a Primary Immunodeficiency Diagnosis: Increasing the Awareness about a Not So Uncommon Pediatric Condition

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Abstract

Background: Primary immunodeficiencies (PID) include a large group of disorders of the immune system that comprise nearly 300 genetic errors of this system. They include a multitude of clinical presentations ranging from the benign asymptomatic immunoglobulin A deficiency to potentially life-threatening diagnoses, such as severe combined immunodeficiency (SCID). PIDs are characterized by an increased susceptibility to infections as well as a predisposition for malignant and autoimmune manifestations due to a dysregulation of the immune system.

Epidemiology and classification: The prevalence of PID in Europe seems to be at least 6 in 100000 inhabitants, although the data provided by different countries vary enormously. They depend strongly on the local capabilities to achieve or not an exact diagnosis, the correct organization of a reporting and registry system, the geographic region and also from the population or ethnicity characteristics. A screening SCID genetic test on newborns has recently been implemented in the USA helping to provide an early diagnosis, which is essential for a successful early therapeutic intervention.

However, there is a general consensus that the number of diagnosed and reported PID is by far lower that their real number. The International Union of Immunological Societies (IUIS) PID expert committee has proposed a detailed PID classification, which is updated every two years in order to include recent information. European Society for Immunodeficiencies (ESID) has also compiled detailed recommendations for PID diagnosis and registry organization. Based on their principal mechanisms, PID is actually grouped into nine disease categories and the assignment of a clinical phenotype to a precise PID diagnosis requires specialized pediatric care.

Conclusions: Patients with a PID may first be presented to a general pediatrician or a generalist, but also many other medical disciplines can encounter this group of patients. Therefore, all physicians handling with general population healthcare and who may lack familiarity with PID need to be aware of this group of diseases in order to use some easy-to-follow algorithms in order to establish a preliminary PID diagnosis and to properly refer these patients to a more specialized health care level. The final correct diagnosis must be provided by a skilled team including at least an immunologist, an infectious disease specialist and also the referring clinician.

Keywords: Primary immunodeficiencies, severe combined immunodeficiency, common variable immunodeficiency, Albanian Population, prevalence of primary immunodeficiencies

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DEFINITION

Primary immunodeficiencies (PID) include a large group of genetic disorders resulting from one or more abnormalities of the immune function and/or regulation (1). Since the first description of the primary agammaglobulinemia in 1952, the number of genetically-defined inborn errors of immunity has increased enormously with nearly 300 gene defects contributing to a multitude of clinical presentations ranging from the asymptomatic IgA deficiency to life-threatening diagnoses, such as severe combined immunodeficiency's (SCID) (2). PID are characterized not only by an increased susceptibility to infections but also by a dysregulation of the immune system functions that may predispose to malignancy, autoimmune diseases, allergy and auto-inflammation (3). PID have served as "experiments of nature in humans" and their study has made possible the detailed knowledge of genes and mechanisms involved in the biology of the immune system.

In many cases, T-cell PID are also associated with B-cell deficiencies in the clinical form of severe combined immunodeficiency (SCID). The patients with SCID express their clinical signs within the first months of life. SCID is characterized by a complete block of T-cell development but also Natural Killer (NK) and B cell number and/or function can be severely affected. Combined immunodeficiency syndromes (CID) have a less severe clinical picture and they are caused mostly by hypomorphic mutations in SCID linked genes or from partial abnormalities of T-cell development. The patients affected by CID express their clinical signs later in childhood by a high frequency of recurrent infections that are often accompanied with a deregulation of the immune system in the form of autoimmunity and/or abnormal lymphoproliferation (4,5).

Over the last few years, impressive progress has been made not only in understanding the disease mechanisms of PID but also in improving the long-term outcomes of potentially curative treatments, including hematopoietic stem cell transplantation (HSCT) and gene therapy (6,7, 8,9). A screening SCID genetic test on newborns has recently been implemented in the USA helping to provide an early diagnosis, which is essential for a successful therapeutic intervention in these patients (10).

PID are indeed more common than generally realized and it is increasingly becoming evident that PID recognized till now constitute only the visible part of a large "iceberg". One factor contributing to the known significant low detection rate of a PID is the low index of suspicion for these diseases, which often derives from a low awareness about this disease group. Another factor influencing the low PID detection rate and also the delay of a PID diagnosis is the lack of availability to carry out systematically all the detailed immunological and genetic laboratory examinations needed to detect the large range of abnormalities affecting the different facets of the immune system. The delay to a PID diagnosis is also related to the

PID type. It is known that common variable immune deficiency (CVID), one of the most frequent PIDs has an estimated delay of 6–8 years in diagnosis after the onset of symptoms and this delay can be more than 3 years for other hypogammaglobulinemias (11).

The rarity of many forms of PID requires national coordination and international collaboration in order to drive improvements in detection, management, and therapeutic strategies and also to facilitate translational research in this field. PID management is resource-intensive, and therefore epidemiological data of high quality are of paramount importance in making health-care planning decisions in this field (12,13).

Epidemiology

Epidemiological data on PID are difficult to obtain and the reports depend strongly on the local capabilities to achieve or not an exact diagnosis, the correct organization of a reporting and registry system, the geographic region, the different methodologies used to register PID and also from the population characteristics, primarily related to the rate of consanguineous marriages in the specific population. Therefore, incidence and prevalence data vary enormously in different reports. For example, in a paper about the PID epidemiology in the UK, Edgar et al report that the incidence of PIDs requiring treatment ranges from 1:20 000 to 1:500 000 depending on the exact diagnosis and the studied population (1). As following the data released by the European Society for Immunodeficiencies (ESID), the minimal incidence in Europe might be around 1:3000 to 1:4000 per year and the prevalence at least 6 for 100,000 inhabitants (13), although the data provided by well documented national studies (14,15,16) and also by ESID Registry (17) show high variability most likely related to deficiencies of data entry in several countries (13). The PID prevalence rates reported in Europe in 2014 by ESID (17) are shown in **Table 1**. They are extremely various and range from 0.056 (Romania) to 6.164 (France) per 100 000 inhabitants. However, there is a general consensus that the number of diagnosed and reported PIDs is far lower that their real number (11,13).

Referring to USA data, the prevalence of any PID diagnosis ranged from 41.1 to 50.5 per 100,000 in the year 2007. The prevalence was two-fold higher among Whites as among Blacks or Hispanics. This study was conducted using a cross-sectional survey in order to estimate the prevalence of PID using related ICD-9 codes (18).

The prevalence rates are different for individual PID, ranging from the most common and often asymptomatic IgA deficiency (1:233 to 1:3000) to the very serious forms of SCID (1:58000) (19). As far as concerns PID requiring treatment, humoral immunodeficiencies are the most frequently encountered and among them common variable immunodeficiency (CVID) has the highest prevalence.

Countries	Prevalence (cases per 100 000 inhabitants)
France	6.164
Spain	4.947
Switzerland	4.182
Netherlands	4.047
Hungary	3.765
United Kingdom	3.705
Estonia	3.059
Turkey	2.355
Czech Republic	2.317
Germany	2.144
Belgium	2.143
Ireland	1.987
Italy	1.955
Greece	1.739
Poland	1.44
Austria	1.178
Slovenia	1.049
Sweden	1.02
Portugal	0.714
Serbia	0.627
Romania	0.056

Table 1. Minimal prevalence rates of PIDs in European countries as reported by European Society for Immunodeficiencies (ESID) (living cases per 100 000 inhabitants in the year 2014) shown in a decreasing order (Reference 18: http://esid.org/Working-Parties/Registry/ESID-Database-Statistics).

Less frequently reported PID categories are predominant T-cell disorders, granulocyte disorders, and the "other well-defined immunodeficiency" syndromes (3, 18,20).

As following the 2014 ESID Registry data, the predominantly antibody disorders are the most frequent PIDs observed (55.66%), followed by "other well defined PIDs" (13.91%), phagocyte disorders (8.73%), predominantly T-cell deficiencies (7.47%), autoimmune & immune dysregulation syndromes (3.89%), auto-inflammatory syndromes (2.06%), unclassified PIDs (1.4%) and defects in innate immunity (1%) (13).

Predominantly antibody disorders are more prevalent in adults than in children and within this category, CVID is the most frequent PID encountered.

Chronic granulomatous disease (CGD) is the most prevalent PID among phagocyte disorders.

In a national study conducted in Tunisia, the estimated prevalence rate was 4.3 per 100,000 inhabitants (21). The prevalence of different PIDs according to the International Union of Immunological Societies (IUIS) classification, was as follows: combined T-cell and B-cell immunodeficiency disorders were the most frequently found (28.6%), followed by

phagocytes abnormalities (25.4%), other well-defined immunodeficiency syndromes (22.7%), predominantly antibody deficiency diseases (17.7 %), diseases of immune dysregulation (4.8%), defects of innate immunity (0.4 %) and complement deficiencies (0.4%). Recurrent infections, particularly lower airway infections (62.3 %), were the most common manifestations in these PID patients.

Diagnostic criteria: How to reach a PID diagnosis?

Suspicion of immunodeficiency is raised when recurrent infections occur in multiple locations and these infections are unusually severe, complicated, and resistant to antibiotic treatment. The infections are characterized by their length and severity and often they are caused by unusual organisms such as *Pneumocystis jiroveci*, *Giardia lamblia* or atypical mycobacteria. The characteristic clinical presentation of PID involves frequent and often severe pulmonary tract infections (recurrent bronchitis and/or pneumonia). Other frequently described symptoms of a PID are intestinal infections. Chronic diarrhea, especially when it is caused by unusual bacteria (e.g., Yersinia or Campylobacter), fungi (e.g., Cryptosporidium), or even common infections such as persistent rotavirus or Salmonella, accompanied by the presence of failure to thrive should trigger an immunologic workup for PID in infants or young children (11).

Other clinical signs include skin lesions (e.g., eczema, warts, abscesses, pyoderma, and alopecia), oral or esophageal thrush, oral ulcers, and periodontitis. In addition to the risk of infection, PID patients have an increased risk for autoimmune diseases, like immune thrombocytopenic purpura (ITP), presumably related to the immune dysregulation. Significantly, there are often delays of many years in the diagnosis of some PIDs, and a late diagnosis increases the risk of chronic organ damage such for example the reported correlation between the delayed diagnosis of X-linked agammaglobulinemia (M. Bruton) and development of bronchiectasis (22).

Recurrent and severe infections of the respiratory tract with encapsulated bacteria like *Streptococcus pneumoniae* and *Haemophilus influenzae* or gastrointestinal infection with *Giardia lamblia* and *Campylobacter jejuni* evoke the presence of an antibody deficiency, whereas recurrent or severe Candida infection, Pneumocystis jiroveci pneumonia and severe viral infections such as cytomegalovirus (CMV), human papilloma virus (HPV) etc. are clinical signs of a T cell deficiency. Recurrent *Neisseria meningitidis* infections are very evocative signs of deficiencies of the common terminal complement components (11).

In order to recognize the children affected by a PID, the clinicians must be very careful to identify the evocative signs as early as possible. In a campaign aiming to educate both families and clinicians, a group of 10 warning signs for PID has been promoted by the Jeffrey Modell Foundation Medical Advisory Board (11). These

warning signs are described in **Table 2**. The difficult issue for the pediatrician is that many normal children can have one or more of these 10 potential warning signs. Therefore, detecting children with a potential PID will require not only a high index of suspicion but also a good clinical experience in order to properly identify whether a child's global clinical presentation is out of the ordinary, compared with other children encountered in the common practice.

A group of investigators has studied the above mentioned 10 warning signs and have concluded that these clinical signs were not developed using evidence-based studies in order to determine their predictive ability (23). They reviewed the records of 430 children who had a definitive PID diagnosis in two immunology clinics in Northern England over a 10-year period. A comparison group included 133 children who presented to these centers with concerns of PID but who did not have PID after evaluation. From the 10 warning signs, they concluded that only 3 of them were significantly predictive of a child having a definable PID: 1 - A positive family history of PID; 2 - Requiring ≥ 2 months of antibiotics without improvement; 3 - Failure to thrive.

Pediatric patients

1. Four or more new ear infections within 1 year.
2. Two or more serious sinus infections within 1 year.
3. Two or more months on antibiotics with little effect.
4. Two or more pneumonias within 1 year.
5. Failure of an infant to gain weight or grow normally.
6. Recurrent, deep skin or organ abscesses.
7. Persistent thrush in mouth or fungal infection on skin.
8. Need for intravenous antibiotics to clear infections.
9. Two or more deep-seated infections including septicemia.
10. A family history of Primary immunodeficiency.

Adult patients

1. Two or more new ear infections within 1 year.
2. Two or more new sinus infections within 1 year, in the absence of allergy.
3. One pneumonia per year for more than 1 year.
4. Chronic diarrhea with weight loss.
5. Recurrent viral infections (colds, herpes, warts, condyloma).
6. Recurrent need for intravenous antibiotics to clear infections.
7. Recurrent, deep abscesses of the skin or internal organs.
8. Persistent thrush or fungal infection on skin or elsewhere.
9. Infection with normally harmless tuberculosis-like bacteria.
10. A family history of Primary immunodeficiency.

Table 2. Ten warning clinical signs for PIDs developed by the Jeffrey Modell Foundation Medical Advisory Board (Reference 11).

No differences in the number of deep-seated infections or episodes of pneumonia or abscesses were found between the children with and without definable PID. Paradoxically, a history of frequent episodes of acute otitis media or sinus infections was associated with a lower risk of definable PID. The conclusion of these investigators is that a PID information campaign must be directed to the hospital pediatricians and also families with a history of PID rather than the general public (23).

LABORATORY EXAMINATIONS

Humoral Immunity

Quantitative and qualitative testing of serum immunoglobulins and specific antibodies can reveal low levels of IgG and/or IgA and IgM, which may be primary or secondary. The significance of a selective low IgM is unclear although it can be associated with aging, autoimmunity, and lymphoproliferative disease. A selective low IgA is found in many apparently healthy individuals, although it may be eventually associated with autoimmunity, allergy and an increased frequency of infections. A low IgG level may represent significant immunodeficiency, especially when associated with a history of unusual or recurrent respiratory or gastrointestinal infections. Very low total serum IgE levels (for example <2 international units/ml) may also represent antibody deficiency. Examination of these serum samples by testing of other immunoglobulin isotypes can lead to a diagnosis of antibody deficiency. Reference ranges of immunoglobulin levels are age-dependent, therefore they levels should be considered carefully in function of age-related normal ranges (24).

Even when a normal level of serum immunoglobulins or a normal B lymphocyte number is encountered, a PID diagnosis implicating a defect in antibody production cannot be excluded. A functional test of antibody production capacity is needed in these cases. At the best, this is carried out by measuring the increase in antibody levels after immunization with harmless vaccines and antigens such as tetanus and/or diphtheria toxoids as well as pneumococcus polysaccharides. A significant increase in these levels 4 weeks after a booster immunization testifies a normal antibody-producing function of B-cells even in the case of the presence of abnormal serum immunoglobulin levels (24). Also, a negative EBV antibody result especially in adults, or an absent or very low antistreptolysin O antibody level even after streptococcus infections should raise the suspicion of an underlying PID.

Patients with PIDs respond poorly to routine vaccinations. Failed vaccine responses, especially when some of them are implicated, are suggestive of an antibody immune-deficiency. When these vaccines contain live organisms, they carry a significant risk of disseminate and severe infection for PID patients especially for those with SCID (25).

The sera of normal individuals contain "natural" anti-A and anti-B blood group antibodies of the IgM isotype, also called iso-hemagglutinins, in correlation to the cell group (eg, a patient with blood group A will have anti-B antibodies, but no anti-A antibodies). The young infants may not have natural anti-A and -B antibodies until approximately 3 months of age, but in other ages, a lack of these antibodies may indicate the presence of an immunodeficiency (24).

Lymphocyte count and function

Persistent unexplained lymphopenia may suggest the presence of a PID particularly in the first few months of life. A low lymphocyte count may be a primary or secondary phenomenon (HIV infection for example) and should prompt further investigation of the lymphocyte subsets and also of serum immunoglobulins in order to exclude a SCID (especially in young children).

Aspects of the immune system (IS) to be examined	Laboratory examinations to be carried out
Counting of cells involved in the IS function (T cell subsets, B cells, NK cells, neutrophils, monocytes, eosinophils)	- Complete blood cell count with manual differential - Blood leucocyte immunophenotyping (IFT) by flow cytometry
Examination of T cell function	- *In vivo:* Intradermal skin test with mitogens (Phytohemagglutinin-PHA) and antigens (Candidin, tetanus toxoid) - *In vitro:* Measurement of lymphocyte stimulation and prolifereation by mitogen (PHA), and antigen (candidin and tetanus toxoid) activation in vitro
Examination of B cell function	- Measurement of serum IgG, IgA, IgM, and IgE levels - Measurement of serum antibody levels to specific antigens before and after vaccination against diphtheria, tetanus, and pneumococcus
Examination of complement system function	- Total hemolytic complement assay (CH50), serum C3, C4 and C1-INH testing
Examination of phagocyte function	- Oxidative burst testing with Nitroblue tetrazolium test (NBT) by microscopy or by Dihydrorhodamine (DHR) test through flow cytometry

Table 3. Initial laboratory workup to be carried out in case of consistent suspicion for a PID diagnosis.

SCID is often associated with a pan-lymphopenia, but selective deficiencies in one or other T-cell subpopulations (e.g., selective CD4 T cell deficiency) can be masked within a normal total lymphocyte count.

The number of lymphocytes in infants and young children are significantly higher than in adults. Therefore, their total levels and also the lymphocyte subpopulations must be considered carefully in relation to the age-appropriate normal ranges (26). However, many PID clinical phenotypes may be accompanied with lymphocyte T subsets within the normal age ranges. In these cases, functional in vivo or in vitro T cell examination by mitogen and antigen stimulation must be performed in order to exclude a T cell deficiency **(Table 3)**.

Phagocyte and monocyte count and function

Although inborn low numbers of polymorphonuclear neutrophils are very rare, abnormal phagocyte functions are not so rare and must be included in the immunological workup of PID **(Table 3).**

Monocytopenia has recently been recognized within a new PID caused by GATA2 deficiency causing a predisposition for atypical mycobacteria and human papillomavirus infections and also a high risk for myelodysplasia and acute myeloid leukemia. Although this condition is extremely rare, a persistently absent or very low monocyte count should be considered carefully.

Testing for complement components such as C3, C4, C1INH or total complement hemolytic assay must also be included in the general immunological workup **(Table 3).**

Platelet count

Idiopathic thrombocytopenic purpura (ITP) can be a presenting sign of primary or secondary immunodeficiency (24). Platelet volume is measured in the normal processing of a full blood count but is usually not reported or considered. A low platelet volume is suggestive of Wiskott–Aldrich syndrome. Although this syndrome is very rare, the finding of a low platelet volume should prompt consideration of this diagnosis. Similarly, autoimmune hemolytic anemia and autoimmune neutropenia can be presenting clinical signs of immunodeficiency such as in CVID for example.

Histopathology Examination

Several histopathological abnormalities may be found in different PID. Granulomatous elements have been found in the granulomatous variant of CVID mimicking similar findings in conditions such as *Mycobacterium tuberculosis* infection, sarcoidosis or Crohn's disease. In the granulomatous variant of CVID, low levels of serum immunoglobulins are usually found. In contrast, in sarcoidosis or other inflammatory diseases, the serum immunoglobulins are expected to be raised (24).

In many immunodeficiency syndromes, notably those involving B cells (like agamma-globulinemias or in the hyper-IgM syndrome), the active germinal centers with their characteristic light and dark zones may be absent or abnormal. If similar findings are detected in a

lymph node biopsy, a PID workup should be prompted. An absence of plasma cells in biopsies can be found in certain PIDs with low or absent B cells, such as X-linked or autosomal recessive agammaglobulinemias, or in a subset of patients with CVID (24).

The finding of a villous atrophy on small bowel biopsy is normally consistent with a diagnosis of celiac disease. However, villous shortening may be associated with infections such as Giardia lamblia which is a common infection found in antibody deficiency.

Genetics Testing

In infants with failure to thrive or with various syndromes, it is often necessary to investigate for chromosomal abnormalities through cytogenetic examination. This test involves a mitogenic stimulation of lymphocytes in order to visualize the metaphase chromosomes. A failed cytogenetic test may indicate a quantitative or functional T cell deficiency and may raise the suspicion for serious PID such as SCID. A delayed SCID diagnosis may lead to life-threatening opportunistic infections by Epstein–Barr virus (EBV), cytomegalovirus (CMV) etc, or to severe infections from normally harmful live vaccines including measles or Bacillus Calmette–Guerin (BCG) (27). An early SCID diagnosis can prevent these infections and can make possible to proceed to a successful HSCT or gene therapy.

A large range of molecular biology tests is actually carried out in specialized labs in order to detect the abnormal genes involved and to properly and definitively establish a PID diagnosis on a genetic basis (28, 29, 30).

Classification

Since the advent of next-generation genomic sequencing, the number of PID-related genetic disorders is increasing quickly every year. The International Union of Immunological Societies (IUIS) PID expert committee has proposed a detailed PID classification, which is updated every two years in order to include the recent information gathered. The PIDs are actually grouped into nine categories based on the principal mechanism of each disease. For each individual PID entity, the genotype and the immunological and clinical phenotypes are also described (31). However, this classification and the respective tables are becoming rather complicated and difficult to be managed. Consequently, this detailed IUIS PID expert committee catalog offers limited assistance to most physicians working at the bedside. In **table 4** we show a modified IUIS PID expert committee classification in a more succinct and summarized form.

Another IUIS expert group, based on clinical and immunological PID phenotypes, has developed some detailed algorithms for each of the 9 groups of PID, in order to reach the diagnosis of a particular PID. These diagnostic algorithms, that are conceived to be used in a tertiary health care level, allow a more rapid and accurate molecular diagnosis and genetic counseling, making possible a more appropriate treatment of affected patients (2).

PID Pathogenetic Categories	Main clinical phenotype features	Inheritance	Number of genetic based disease diagnoses	Prevalence rate among all PIDs (cases per 100 000 inh.)
1. Combined T and B cell PIDs (Predominantly T cell)			Total: **50**	0.05 - 0.52
	1. T – B+ SCID	XL or AR	8	
	2. T – B- SCID	AR	8	
	3. CID less profound than SCID (can be presented as T+B+ CID)	XL or AR	34	
2. Combined ID with associated or syndromic features Wiskott-Aldrich; Di George; Ataxia-telangiectasia etc.	10 different clinical entities (can be presented as T+B+ CID)	XL or AR	Total: **45**	0.24 - 0.64
3. Predominantly antibody deficiencies (4 groups)			Total: **34**	1.27 - 2.93
	1. Severe reduction of all Ig isotypes and profound decrease or absent B cells	XL or AR or unknown	9	
	2. Severe reduction in at least 2 serum Ig isotypes and presence or low numbers of B cells	AR, AD or variable	12	
	3. Severe reduction in serum IgG and IgA with normal/elevated IgM and normal numbers of B cells	AR	4	
	4. Isotype or light chain deficiencies with generally normal numbers of B cells	AD, AR, or variable	9	
4. Diseases of immune dysregulation			Total: **37**	0.05 - 0.31
	1. Familial hemophagocytic lymphohistiocytosis (FHL) syndromes	XL, AR	10	
	2. T regulatory cells genetic defects	XL, AR, AD	4	
	3. Autoimmunity with or without lymphoproliferation	AR, AD	9	
	4. Immune dysregulation with colitis	AR, AD	4	
	5. Type 1 Interferonopathies	AR, AD	10	
5. Congenital defects of phagocyte number, function, or both			Total: **29**	0.16 - 0.72
	1. Congenital neutropenias	XL, AR, AD	15	
	2. Defects of Motility	XL, AR, AD	9	
	3. Defects of Respiratory Burst	XL, AR, AD	5	

6. Defects in Intrinsic and Innate Immunity			Total: **33**	0.0 - 0.14
	1. Mendelian Susceptibility to Mycobacterial Disease (MSMD)	AR, AD	11	
	2. Epidermodysplasia verruciformis (EVER def; WHIM syndr.)	AR, AD	3	
	3. Predisposition to severe viral infection (STAT, IRF 7, CD16 def.)	AR	4	
	4. Herpes simplex encephalitis (TLR3, TRAF3, TRIF def)	AD, AR	5	
	5. Predisposition to invasive fungal diseases (CARD9 def.)	AR	1	
	6. Chronic mucocutaneous candidiasis (IL17, STAT1, ACT1 def.)	AD, AR	5	
	7. TLR signaling pathway deficiency (IRAK4, MyD88 def) (Bacterial infections)	AR	2	
	8. Isolated congenital asplenia (RPSA mutation)	AD	1	
	9. Trypanosomiasis (APOL-1 mutation)	AD	1	
7. Autoinflammatory disorders			Total: **21**	0.01 - 0.06
	1. Defects affecting the inflammasome (FMF et.)	AD, AR	9	
	2. Non inflammasome-related conditions (TRAPS syndr. etc)	AD, AR	12	
8. Complement deficiencies			Total: **30**	0.02 - 0.33
	1. Integral complement cascade component deficiencies (C1q – C9 def)	AD, AR, XL	19	
	2. Complement Regulatory defects (C1INH etc)	AD, AR, XL	11	
9. Phenocopies of PID			Total: **10**	
	1. Associated with somatic mutations		4	
	2. Associated with autoantibodies		6	
TOTAL			**289**	**2.0 – 6.0**

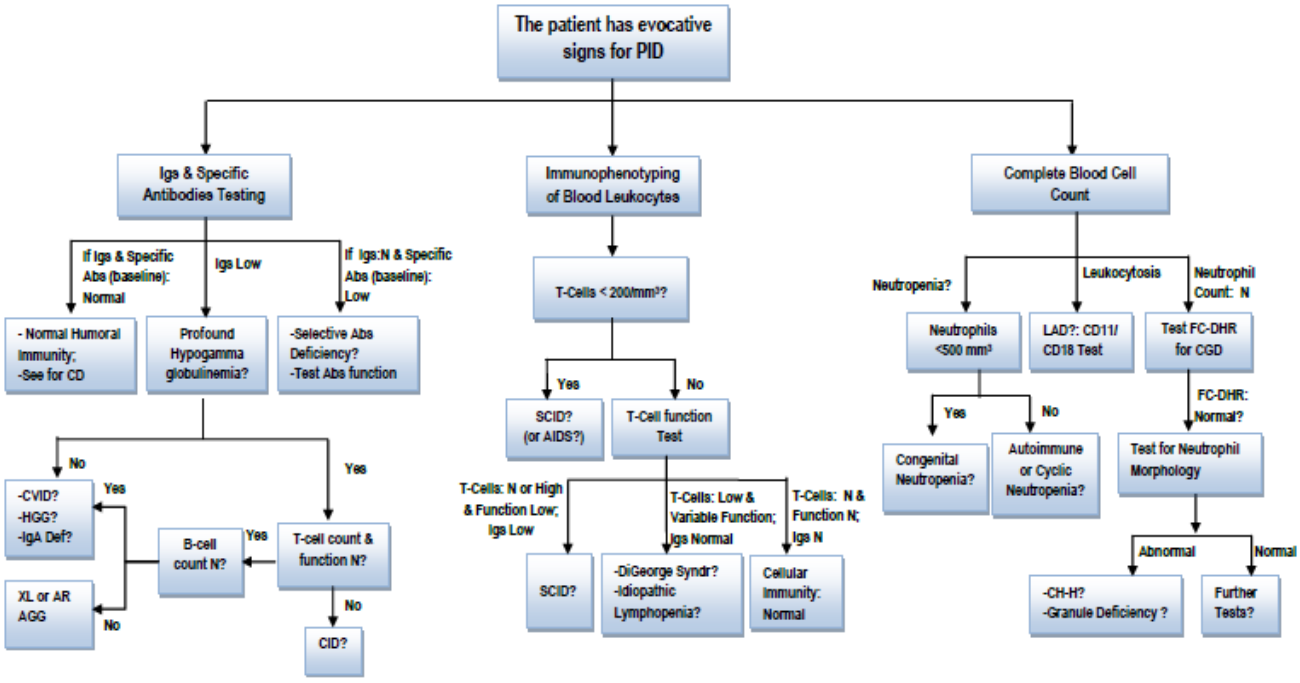
PID – primary immunodeficiency; **SCID** – severe combined immunodeficiency; **CID** – combined immunodeficiency; **XL** – X-linked; **AR** – autosomal recessive; **AD** - autosomal dominant

Table 4. PID IUIS 2015 classification (Ref. 32) modified in a shortened version.

However, patients with PID related clinical signs are usually first presented to a general practitioner or pediatrician not specially trained in PID diagnosis. Especially for those outside the field of PID, those in training or for the general clinicians, a more simplified clinical diagnostic algorithm is needed (32).

Such physicians need an easy-to-follow diagnostic scheme that is based on the clinical and/or biological phenotype that they observe. In order to reach a rapid and preliminary PID diagnosis, a practical scheme has been proposed to be used by the general pediatricians through a relatively simple diagnostic algorithm (11). We have modified this scheme in **Figure 1** in order to condense the diagnostic algorithm in a unique figure.

Figure 1. Schematic presentation of a simplified workup algorithm for primary immunodeficiency diagnostics (modified from Lehman et al; Ref. 11).



Abs – Antibodies; **AIDS** – Acquired Immune Deficiency Syndrome; **AGG** – Agammaglobulinemia; **AR** – Autosomal Recessive; **CD** – Complement Deficiencies; **CGD** – Chronic Granulomatous Disease; **CH-H** – Chediak-Higachi Syndrome; **CID** – Combined Immune Deficiencies; **CVID** – Common Variable Immunedeficiency; **Def.** – Deficiency; **FC-DHR** – Dihydrorhodamine Assay by Flow Cytometry; **Igs** - Serum Immunoglobulins; **HGG** – Hypogammaglobulinemia; **LAD** – Leukocyte Adhesion Deficiency; N- Normal; **PID** – Primary Immunodeficiencies; **SCID** – Severe Combined Immune Deficiencies; **XL** – X-Linked

Data from the Laboratory of Immunology of the University Hospital Center of Tirana

In order to obtain a preliminary estimation of the PID prevalence in Albania, we examined the immunological examination results of 1737 pediatric patients that have been tested in our laboratory for their IgG, A and M serum levels and/or peripheral blood leukocyte subpopulations during a 5 year period (2010-2015). Taking into account that the Laboratory of Immunology of the University Hospital Center of Tirana is the reference immunological laboratory for all Albania, we can consider that most if not all patients with a PID suspicion are sent for examination in this center. From all patients tested during the 5-year time lapse, we detected 40 cases with abnormal serum immunoglobulins and/or lymphocyte subpopulations results accompanied by recurrent infections and without a known primary cause.

Among them, 18 cases were found to be with isolated IgA deficiency, 7 with Bruton agammaglobulinemia (total absence of immunoglobulins and B cells), 4 with isolated IgM, 3 with low IgA and IgM, 3 with low IgG and IgA, 2 cases with low IgG and IgM, 2 cases with isolated IgG and 1 case was diagnosed with SCID. No PID with phagocyte, innate immune system, complement or other dysregulation /autoinflammatory disorders have been detected. The positivity detection rate was 2.3 % and a probably detected prevalence rate can be tentatively proposed at approximately 1.4 cases per 100 000 inhabitants. The real prevalence rate must be indeed higher due to a probable low diagnostic rate of some PIDs in our country. This low detection rate seems to concern mainly the PIDs due to cellular causes (T cell and phagocyte cells) that have a more rapid and severe disease course and that require an early, rapid, and detailed diagnostic workup.

CONCLUSIONS

The consequences of a delayed diagnosis of a PID are recurrent, severe and potentially life-threatening infections and/or chronic organ damage (eg, bronchiectasis). Detailed diagnostic immunologic and genetic examinations must be performed as soon as a PID diagnosis is suspected in these patients. An early and detailed diagnosis will aim to prevent the occurrence of irreversible damages and will make possible appropriate and timely interventions in order to prevent chronic morbidity and mortality (33).

All patients with a PID suspicion must be addressed to a centralized tertiary care pediatric center where they must be submitted to a thorough PID workup based on the clinical algorithms described above. The final correct diagnosis must be provided by a skilled team including at least an immunologist, an infectious disease specialist and also the referring clinician. This center must organize a centralized PID registry conforming to the ESID guidelines (13, 17). Early interventions with regular intravenous immunoglobulins will prevent irreversible complications (20,34,35).

In the case of a cellular PID (abnormal T cell or phagocyte function), the possible interventions (HSCT or gene therapy) are successful if performed within the first months of life (6,7,8). Obligatory newborn BCG vaccination must be critically evaluated in case of families with a PID history because they can be life-threatening in the eventuality of the presence of a T-cell PID.

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Postmortem Acute Death Obtained Brains without Reperfusion are Utilizable and Reliable to Examine the Expression of Hypoxia Related Antigens

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Abstract

Background: It is widely accepted that the Purkinje cells in the cerebellum and the cells in the hippocampus are very vulnerable and sensitive to hypoxic circumstance. Many researchers believe firmly that the postmortem tissues are not suitable to investigate signal transduction in the cells, especially neurons.
Aim: However, we presumed that the vulnerability might be result from reperfusion occurred in the brain during agonal duration. Then, using by means of immunohistochemical method we examined the expression of hypoxia related antigens in the hippocampus, the cerebellum and the thalamus obtained from two individuals who acutely died due to self-strangulation without reperfusion of blood.
Methods and Results: We examined morphological changes and expression of hypoxic related antigens using conventional and immunohistochemical techniques. There was no remarkable damage such as pyknosis of the cell-nuclei, and vacuolation of the cell-cytoplasm by HE stain except weak edematous space around the cells at light microscopic level. The antibodies against hypoxic related antigens showed reactivity with the cell cytoplasm or nuclei corresponding to their specificity. Although, anti Hypoxia inducible factor 1 alpha (HIF1α) antibody known as the most famous one to detect hypoxic situation in the tissues showed very weak or feeble reactivity, antibodies against RNR binding protein motif 3 (RBM3), cold inducible RNA binding protein (CIRBP), endothelial nitric oxygenase synthase (eNOS), vascular endothelial growth factor (VEGF) and Heat shock protein 70 (HSP70) showed better reactivity than that by HIF1αin those cells. The antibodies against apoptosis related antigens such as Apoptosis inducible factor (AIF), and tumor protein p53 (p53) showed feeble reactivity with these cells. The reactivity of anti eNOS antibody showed strange results among the brain regions.
Conclusions: The results obtained from present study may indicate that the brains obtained from acute death individuals keep its morphology and are in the signal pathway situation to protect hypoxic brain damages and not in a process from apoptotic to cell death.

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The brain tissues without blood reperfusion might be utilize and reliable to evaluate and examine the cell functions at microscopic level.

Keywords: Hypoxic brain damages, Hypoxia related antigens, Acute hypoxic changes, Immunohistochemistry.

INTRODUCTION

It has been widely accepted that self-ligature strangulation was not able to make one's death, since lowering of consciousness occurred due to stopping of blood supply to the brain and subsequently the ligature was loosen when one would perform squeezing one's neck surface without apparatus for fixing the ligature. However, it is a fact that in forensic death investigation cases we have experienced and encountered self-strangulation death cases. We indicated in a previous report (1) that when one could keep the tension of a ligature at 5 Kg that is essential force to obstruct the cervical artery, after losing one's consciousness, two routes of blood stream to the brain may be stopped, and the tension with 2 Kg that is essential force to obstruct the internal jugular vein, could obstruct return-way to the heart and increase intracranial pressure. And we also described that the death mechanism including physiological- and anatomical-perspective view in a previous report (2).

As a confirmation of our assumption concerning with performing self-strangulated death, Morita et al (3), reported a rare case in which a 71 year-old man was found dead on his bed, and it was concluded by police investigation that he died due to stopping of returning of venous blood to the heart by compression of his neck-surface with a string drawn by a dumbbell with 3kg in weight. It is well known that the brain is particularly susceptible to interferences with its blood supply. In the absence of blood flow, and therefore of oxygen, the energy reserves of the brain are capable of sustaining ATP levels for about 1 min (4). We experienced one video recorded hanging case where a man attempt hanging and died within three minutes after his performance hanging (5).

In the adult human brain, acute hypoxic episodes result in a certain pattern of neuronal cell damage from which a hierarchy of neuronal vulnerability can be formed. Among the most sensitive regions are the "older" brain structures like hippocampus and cerebellum. In these structures, the typical picture is loss of pyramidal and Purkinje cells. Also, the neo-cortex is among the vulnerable structures, and often a characteristic laminar neuron loss is found. (6). Recently, it has been described that the Purkinje cells in the cerebellum and the cells in the hippocampus are very vulnerable and sensitive to hypoxic situation. However, the changes of these cells were observed in the cases where the death occurred after one day of event occurrence and re-perfusion of the brain was usually accompanied (7). In addition, these describing concerning to the neuronal damages were limited in cases where reperfusion in the brain occurred.

In our experience the level of pathological damaging of the granular cell layer of the cerebellum was severely in a hanging death with 24 hours reperfusion than those of a killed female by manual strangulation case in which her body was buried in a garden for 42 days (8).

Since no study has examined the expression of hypoxia related antigens in the hippocampus and cerebellum of individuals who acutely and directly died due to self-strangulation, we examined the expression patterns of the hypoxic, apoptotic related antigens in the cerebellum and the hippocampus obtained from two victims who died due to self-strangulation using a T-shirt or a cotton rope (2).

MATERIALS AND METHODS

Slice of the hippocampus, thalamus and the cerebellum containing dentate nucleus from two victims at autopsy, Department of Legal Medicine, Shiga University of Medical Science. After fixed by 10% of formalin, paraffin embedded blocks were prepared and each block was cut with 3 μm to mount on a slid-glasses. Non-specific binding was blocked for 1 h in suitable serum. The slides were incubated with different antibodies overnight at 4 C. The antibodies used in this study were listed in **Table 1**. Biotinylated antibodies and the avidin-biotin complex were applied for 90 min.

Table 1. Characteristics of the antibodies used in this study

Antibody	Maker	Clone	Species	Antigen-retrieval	incubation	Antibody-dilution
CIRBP	Protein Tech	10209-2-AP	Rabbit	autoclave	overnight	1:400
RBM3	Protein Tech	14363-1-AP	Rabbit	autoclave	overnight	1:400
HSP70	santa cruz	polyclonal	Goat	autoclave	overnight	1:400
HIF-1α	Novus	NB100-479	Rabbit	autoclave	overnight	1:400
VEGF	Milipore	JH121	Mouse	autoclave	overnight	1:400
eNOS	Gene Tex	polyclonal	Rabbit	autoclave	overnight	pre-diluted
AIF-α	LSBio	aa-593-606	Rabbit	autoclave	overnight	1:400
P53	santa cruz	FL-393	Goat	autoclave	overnight	1:400
cFOS	Gene Tex	polyclonal:	Rabbit	autoclave	overnight	1:800
Ngb	SIGMA-ALDRICH	polyclonal	Rabbit	autoclave	overnight	1:400
Wnt	Novus	6F2	Mouse	autoclave	overnight	1:400
SIRT1	Novus	E104	Rabbit	autoclave	overnight	1:400
CCC9	Leica	10A6	Mouse	autoclave	overnight	1:400

Finally, diaminobenzidine (DAB) was added fro 5 min for visualization. At each step, slide glasses were washed by phosphate buffered saline. Slides were dehydrated, dried, and covered with a cover glass.

RESULTS

1. HE staining

There was no remarkable decreasing of number of cells in granular cells and morphological changes of the cell nuclei in the cerebellum and hippocampus dentate gyrus. The same morphological situation was observed in other region of hippocampus containing C1 to C4 regions, the cerebellum containing the Purkinje cells and dentate nucleus and the neurons in the thalamus from two individuals.

2. Immunohistochemical staining

2-1 The hippocampus

Antibodies against RBM3, CIRBP, e-NOS, HSP70, VGEF and Neuro globurin (Ngb) could stain the cells in granular cells of dentate gyrus, and the cells in C1 to C4 regions showing different stainability by each antibody. For example, anti RBM3 and CIRBP stained nuclei with clear and intensive reactivity, and other antibodies such as VGEF, HSP70 and e-NOS stained cytoplasm of the cells with moderate reactivity. Anti Ngb showed weak reactivity with cytoplasm. On the other hands, antibodies against HIF1, SIRT1, p53, AIFα, and CCC9 showed no reactivity in these cells. The staining results of the granular cell layer are shown in the **Photo 1.**

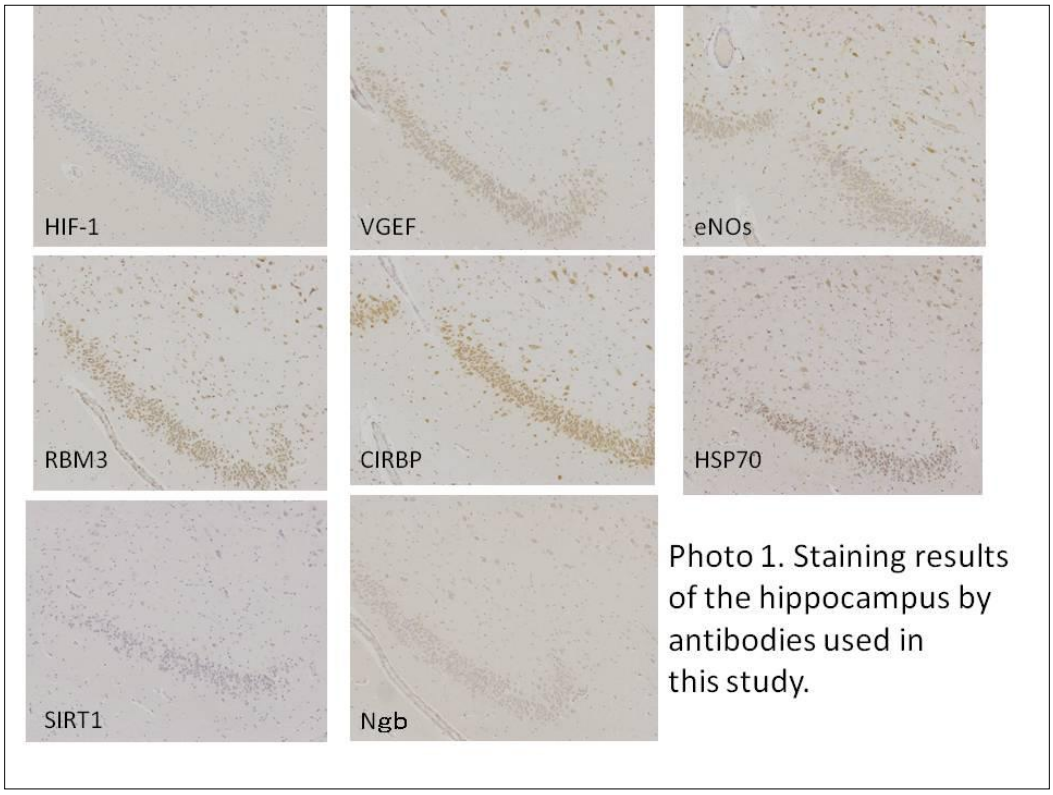


Photo 1. Staining results of the hippocampus by antibodies used in this study.

2-2 The thalamus

Antibodies against RBM3, CIRBP and eNOS showed clear and good reactivity with the nuclei or cytoplasm of neurons in the thalamus. Antibodies against HSP70, and VEGF showed weak or moderate reactivity with the neurons. Antibodies against SIRT, HIF1α, p53, AIF and CCC9 showed feeble reactivity with neurons in the thalamus.

2-3 The cerebellum

Antibodies against CIRBP and RBM3 showed clear reactivity with the cells in the granular cell layer and anti HSP70 antibody showed weak reactivity with these cells. However antibodies against HIF1α, SIRT 1, Ngb, cFOS and P53 showed no reactivity with these cells.

The Purkinje cells showed intensive reactivity with CIRBP and RBM3 in the nuclei and weak reactivity with Ngb, c-FOS, CC9 and P53 in cytoplasm of them. No reactivity was observed with antibodies against HSP70, HIF1α, SIRT1 and eNOS in the Purkinje cells. There was one different in the stain-ability of the Purkinje cells between anti RBM3 and CIRBP antibodies. Anti RBM3 reacted only with nuclei and anti CIRBP reacted both with nuclei and cytoplasm of the Purkinje cells. The cells in the molecular layer showed intensive with anti CIRBP and RBM3 antibodies, moderate with antibodies against CC9, SIRT1, HIF1αand HSP70. Anti HSP70 showed clear reactivity with the cells in the Purkinje cell line.

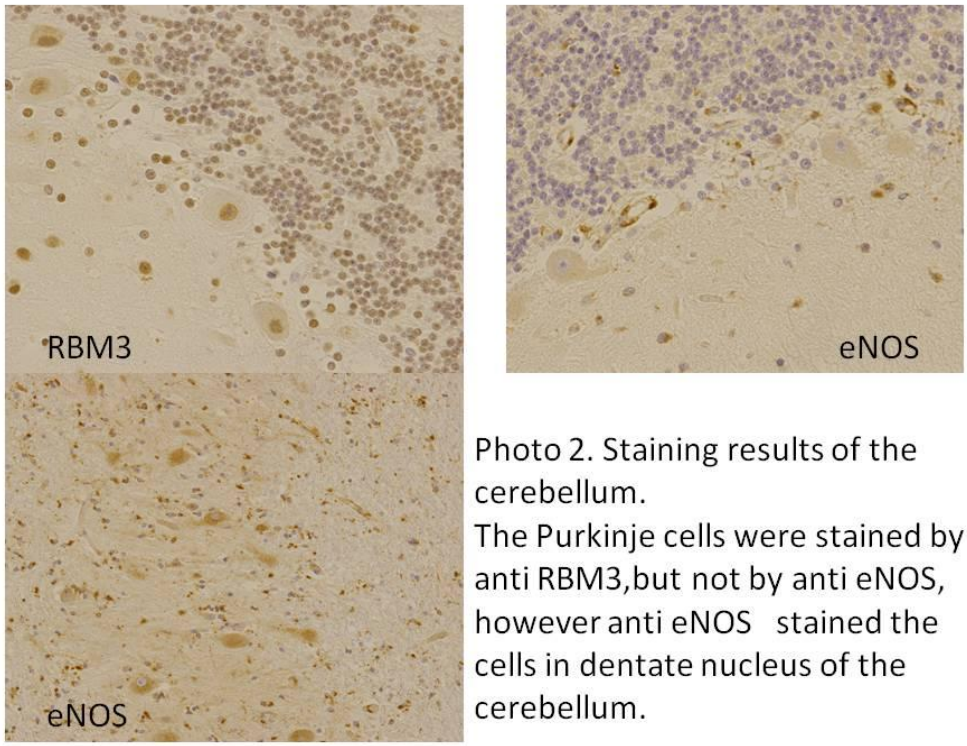


Photo 2. Staining results of the cerebellum.
The Purkinje cells were stained by anti RBM3,but not by anti eNOS, however anti eNOS stained the cells in dentate nucleus of the cerebellum.

Regarding to the cells in the dentate nucleus all antibodies except anti SIRT1, AIF1 and VEGF antibodies showed reactivity in a variety of stain-ability. Although anti eNOS showed no reactivity with Purkinje cells and cells of molecular layer, this antibody showed clear reactivity in the cells of the dentate nucleus as shown in **Photo 2** with extension of the reaction time at each step.

3. Choroid plexus

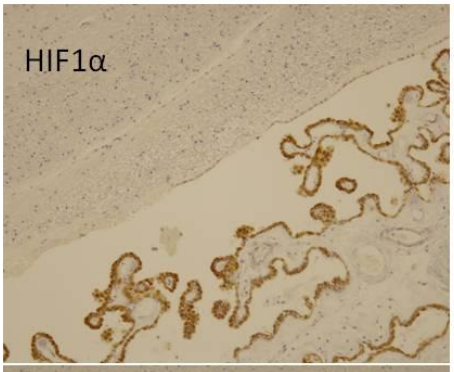
In the choroid plexus the staining results were dissociated by those obtained from other regions. Although the staining intensity was different among the antibodies, all antibodies showed good reactivity with the cell in the choroid plexus. Staining results were shown in **Photo 3**.

4. Summary of staining results

Each region collected from brains of individuals who died due to self-strangulation has kept stain-ability by antibodies against hypoxic related antigens although the staining intensity was different among the antibodies. The stainability of each antibody was similar between two individuals.

DISCUSSION

The present and previous observations (8) with HE stain indicate that brain tissues obtained from acute deaths without reperfusion of blood stream are reliable and useful to examine histochemical characteristics of human post-mortem brain.



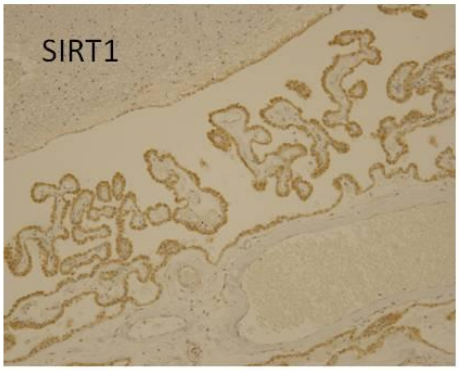


Photo 3. Staining results of choroid plexus.
All antibodies used in this study could stain the cells in the choroid plexus.

The brain is the organ that is most susceptible to a variety of hypoxic insults such as are brought about by cardiac arrest, status epilepticus and hypoglycemia in clinical medicine (9). In addition to them, there are many insults such as drowning, chest- and neck-compression and anaphylaxis shock in forensic field.

We previously studied the expression of hypoxic related antigens such as HIF1α, SIRT1, RBM3, CIRBP, e-NOS, HSP70, VGEF and Ngb in the thalamus obtained from two case of self-ligature strangulation death (2).

Malhotra et al (10) and Carmeliet et al (11) reported that HIF1 played a master regulatory role in the cellular response to hypoxia. In certain circumstances and in certain cell types, HIF1 promoted apoptosis in the presence of hypoxia, especially when other cellular energy substrates were lacking. It is now well accepted that hypoxic-ischemic brain damage following asphyxia and/or blood flow disruption occurs in a biphasic manner, which are necrosis and apoptosis (12). Since the decubitus ulcer does not occur during postmortem interval, the decubitus ulcer occurs in reperfusion of blood supply after sever compression of the skin, during reperfusion with progressing of the cascade of signal pathway, apoptosis or necrosis of the skin become to be reliable and be remarkable.

Dalkara et al (13) reported that enhanced NO production within the cerebral vasculature protects brain tissue during focal ischemia via hemodynamic mechanisms whereas neuronal overproduction may facilitate or mediate neurotoxicity. Samdani et al (14) described that eNOS plays a prominent role in maintaining cerebral blood flow and preventing neuronal injury. Bolanos JP and Almerida A (15) reported that activation of nNOS or induction of iNOS mediates ischemic brain damage, possibly by mitochondrial dysfunction and energy depletion. However, eNOS activation within the endothelium of blood vessels mediates vasodilatation and hence increases blood flow to the damaged brain area. Although few works show e-NOs location in the cerebellum, studies by Hernandez et al (16) and Shin et al (17) have found e-NOs in Purkinje cells and neurons of the cerebellum nucleus. Iwase et al (18) suggested that brain eNOS is involved in early pathophysiological response against systemic infection before iNOS is induced with progression of the infection. In the present study anti eNOS antibody showed intensive reactivity with the cytoplasm of cells in the dentate nucleus of the cerebellum, however feeble reactivity was detected in the Purkinje cells. This phenomenon is not consistent with the reports described above. The expression of eNOS might not detected due to delay of signal cascade in cells, since the expression of eNOS was related with the expression of HIF1α (19) and the feeble expression of HIF1α was observed in this study. It is well accepted that HIF1 which closely relate with the prevention of hypoxic control and manages to expression of VEGF, and the expression of VEGF is

connected with the expression of e-NOs, since Qing et al (19) have proposed that the response to hypoxia is primarily mediated by the transcription factor hypoxia-inducible factor-1 (HIF-1) which leads to the induction of a variety of adaptive gene products including VEGF and eNOS.

In our examinations indicated that expression manner between RBM3 and HIF1α was different in the substantia nigra (20, 21) obtained from same individuals and expression of RBM3 was more intensive compared with that of eNOS (22), showing expression of RBM3 was independent of cascade in HIF1α pathway.

Although the signaling cascades upstream and downstream of RBM3 and CIRBP remain to be elucidated, the up-regulation of RBM3 and CIRBP in the case of hypothermic (23) and hypoxic/ischemic deaths may rescue neuronal cells from apoptosis. Smart et al (24) reported that RBM was expressed in multiple brain regions, with the highest levels in cerebellum and in dissociated neurons RBM3 was observed in nuclei and in a heterogenous population of granules within dendrites, and RBM3 plays a distinctive role in enhancing translation in neurons. In our experiences no expression of RBM3 was observed in the nuclei inside the acute infarcted area of the myocardium (25) and nuclei in the substantia nigra cells from the victims with cirrhosis or brain crush (22). Regarding to expression of RBM3 and eNOS, we experienced that although both antigens could be detected in ischemic area of the cardiac tissue where located surrounding of cardiac infarction area, weak reactivity with anti eNOS antibody was observed and no reactivity with anti RBM3 was recognized inside the infarction area (25). This experience indicate that expression of both antigens are also independent each other.

The future study will be undertaken with the aim of answering the question; is the expression pattern of antigens examined in this study in C1 to C4 areas, dentate gyrus of the hippocampus and in the cerebellum similar or different compared with those of the basal ganglia and the hypothalamus?

SUMMARY

The degree of damages occurred in the brain cells due to ischemic is based on the period of agonal duration and re-perfusion. The brain tissues obtained from acute and direct death individuals may be appropriate to examine the cascade and/or signal pathway in the cells, since many antigens, such as eNOS, RBM3, ICRBP, VGEF, and HSP, were expressed in the hypoxic situation even in the hippocampus and/or the Purkinje cells known as the most vulnerable regions in the brain, and no or feeble expression of apoptotic and inflammatory antigens were observed in those regions.

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Link between Biochemical and Hematological Parameters and their Role as Pre-diagnostic Indicators of Acute Inflammation in Preschool Children

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Abstract

Background: Correlations between biochemical and hematological parameters and acute immune response in children are insufficiently described. The aim of this study was to determine the potential links between selected biochemical and hematological parameters and the development of any type of inflammation in children of preschool age, as well as to determine the concentration ratio between C-reactive protein and serum iron.

Patients and methods: The subjects of this research were preschool children suspected of inflammation in the municipality of Novo Sarajevo. All biochemical parameters (C-reactive protein, total iron binding capacity, unsaturated iron binding capacity and serum iron) were determined using a Cobas® 8000 automatic analyzer. Hematological parameters (white blood cell count, hematocrit, hemoglobin concentration, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration) were determined using an automated hematology analyzer Celltac F®.

Results: Iron levels were reduced in children who had elevated levels of C-reactive protein. White blood cell counts did not significantly differ from reference limits ($p>0.05$). Hematological parameters (HCT, hemoglobin concentration, MCV, MCH and MCHC) did not deviate from reference values in cases of acute inflammation and therefore cannot be used as valid indicators of inflammation in preschool children. Gender-specific differences have not been established for any parameter.

Conclusion: Elevated levels of C-reactive protein in combination with serum iron levels are the best indicator of acute inflammation in preschool children. TIBC and UIBC together with serum iron levels might be a prognostic marker of compensatory anemia development.

Keywords: C-reactive protein, serum iron, acute inflammation, preschool children.

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INTRODUCTION

The sensitivity of child's organism causes slightly different responses to the acute phase of inflammation. The correlation between biochemical and hematological parameters during acute inflammation is still subject of discussion.

C-reactive protein (CRP) is synthesized in the liver in response to a variety of inflammatory processes in the organism. It is an acute phase reactant and its principal role is the activation of complement and the initiating of an immune response. CRP synthesis is induced by cytokines, interleukin-6 (IL-6), interleukin-1 (IL-1) and indirectly by tumor necrosis factor alpha (TNF-α) (1,2). The synthesis of CRP starts very fast and reaches a maximum within 48 hours. During the acute inflammatory reaction, CRP values can be increased up to hundred times. Reference values for CRP are under 5 mg/L (3). Plasma contains about 2.5 mg of iron which is transported bound to a plasma protein transferrin (4). UIBC is a parameter of latent or unsaturated iron-binding capacity while TIBC shows the maximum concentration of iron that transferrin can bind. Reference values for serum iron in preschool children range from 10 to 30 μmol/L and for TIBC varies between 43 and 80 μmol/L (5,6). The number of leukocytes is a dynamic equilibrium between the bone marrow formation and their deterioration and depends on the physiological state of the organism. The number of leukocytes in preschool children ranges from 5.3 to 11.5 x 10^9 per liter of blood (7). Reference values for hematological parameters in preschool children are: hemoglobin 11.5 to 13.5 g/dL , HCT 31.7 to 39.6%, MCV 72.7 to 86.5 fL, MCH 24.1 to 29.4 pg and MCHC 32 to 35.3 g/dL (7,8,9).

The aim of this study was the assessment of correlations between biochemical and hematological parameters and acute inflammation reactants in preschool children, with special emphasis on the correlation between CRP and other biochemical or hematological parameters.

PATIENTS AND METHODS

Subjects

This research was conducted on preschool children from the municipality of Novo Sarajevo (Bosnia and Herzegovina). They were born between 2009 and 2012. Blood samples were drawn between 7 and 10 o'clock in the morning, after 15 minutes of inactivity (Department for Laboratory Diagnostics, Health Center "Omer Maslić", Sarajevo, Bosnia and Herzegovina).

Our research was conducted in compliance with all applicable guidelines with ensured proper implementation of the safety of persons participating in the scientific research, including Fundamentals of Good Clinical Practice, Declaration of Helsinki 1975, as revised in 2008, and the Law on rights, obligations and responsibilities of patients in Federation of Bosnia and Herzegovina. Analysis was carried out with the consent of all parents.

This study covered pre-school children with symptoms of acute infections e.g. fever, sore throat, malaise, poor appetite–symptoms caused by bacterial or viral infections. The cause of infection varied as shown by analyzed parameters, especially with the values of CRP.

Sampling

The blood for analysis was obtained from median cubital vein. In samples for hematological analysis was added anticoagulans (EDTA) while blood samples for biochemical analysis (including CRP) didn't contain anticoagulans.

Biochemical analysis

Biochemical parameters (CRP, total serum iron, UIBC and TIBC) were determined with the automatic autoanalyzer Cobas® 8000 (model Cobas c502, Roche Diagnostics, USA). After the preparation procedures, samples were put into the autoanalyzer and the concentrations were measured by turbidimetric or spectrophotometric methods. C-reactive protein concentration was determined by the autoanalyzer Cobas c 502 (imunoturbidimetric principle) (10,11). UIBC and serum iron levels were obtained by FerroZine method (12,13).

Hematological analysis

Hematological parameters included total number of leukocytes (WBC), hematocrit (HCT), hemoglobin (Hb), mean corpuscular volume (MCV), mean concentration of hemoglobin in erythrocytes (MCH) and mean corpuscular hemoglobin concentration(MCHC).

All parameters were analysed by autoanalyzer Celltac F® (Nihon Kohden, Japan). The analysis were based on flow citometry principles (14).

Statistical analysis

Statistical methods included analysis of variance (ANOVA), Spearman bivariate correlation and Kolmogorov-Smirnov test. Statistical analysis were performed by SPSS(Version 20.0, SPSS, Inc., Chicago, IL, USA) and MS Excel 2013 statistical program. P values lower than 0.05 ($P<0.05$) were considered as significant and P values lower than 0.01 ($P<0.01$) as highly significant.

RESULTS

This study included 51 children (29 boys and 22 girls). C reactive protein (CRP) concentration, hematocrit (HCT), hemoglobin concentration (Hb), leukocytes (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), serum iron concentration (Fe), total iron binding capacity (TIBC) and unsaturated iron binding capacity (UIBC) were determined in all individuals.The results of biochemical and hematological analysis (including mean and standard deviation) are shown in **Table 1** and compared with reference ranges.

Parameter (n = 51)	Reference range	Mean ± SD (n = 51)	♂♂ (n = 29)	♀♀(n = 22)	P-value ♂♀
CRP (mg/L)	0-5	29.63 ± 52.35	21.03 ± 35.50	40.95 ± 67.89	<0.05*
Fe (µmol/L)	10-30	7.18 ± 5.64	6.98 ± 5.06	7.44 ± 6.44	>0.05
UIBC (µmol/L)	33-50	49.87 ± 9.94	51.07 ± 9.33	48.30 ± 10.71	>0.05
TIBC (µmol/L)	43-80	57.02 ± 8.35	58.14 ± 7.67	55.55 ± 9.15	>0.05
WBC (x 10^9/L)	5-12	10.47 ± 4.13	10.85 ± 3.77	9.96 ± 4.59	>0.05
Hb (g/dL)	12-14	12.2 ± 1.27	12.31 ± 1.33	12.04 ± 1.21	>0.05
Hct (%)	32-40	35.15 ± 5.57	36.17 ± 3.44	35.30 ± 3.16	>0.05
MCV (fL)	73-87	75.81 ± 4.45	75.35 ± 4.54	76.40 ± 4.34	>0.05
MCH (pg)	24-30	25.74 ± 1.87	25.64 ± 1.92	25.16 ± 1.85	>0.05
MCHC (g/dL)	32-35	33.99 ± 1.00	34.01 ± 0.82	33.96 ± 1.22	>0.05

*P<0.05 is statistically significant

Table 1. Biochemical and hematological parameters of preschool girls and boys accompanied by gender specific analysis and P-values.

Parameter	Kolmogorov-Smirnov (df = 51)		Spearman correlation coefficient	
	Statistics	Sig.	CRP	
CRP	0.292	0.00.**	F	Sig.
Fe	0.236	0.00.**	-0.523	0.00.**
UIBC	0.076	0.200	0.175	0.22
TIBC	0.143	0.011*	-0.116	0.418
WBC	0.135	0.021*	0.354	0.011*
Hb	0.064	0.200	-0.497	0.00.**
Hct	0.086	0.200	-0.432	0.002**
MCV	0.103	0.200	-0.315	0.024*
MCH	0.124	0.047*	-0.492	0.002**
MCHC	0.102	0.200	-0.492	0.002**

* P<0.05 is statistically significant

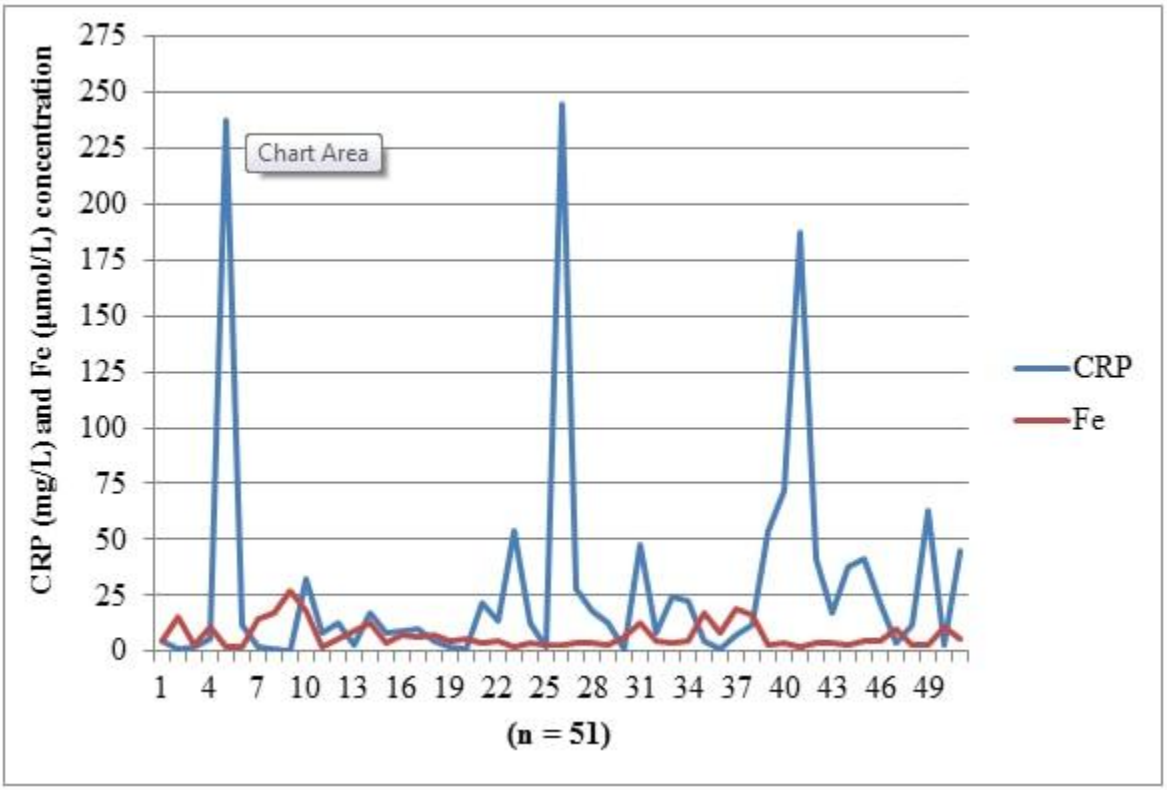
** P<0.01 is statistically significant

Table 2. Results of Spearman bivariate correlation test between CRP and other parameters and Kolmogorov-Smirnov normality test.

The highest deviation in comparision with reference values was obtained for CRP (29.63 ± 52.35 mg/L) and for the concentration of serum iron (7.18 ± 5.64μmol/L). Other parameters were within reference ranges. Boys had higher values of obtained parameters but without significant differences in comparison to girls, except for CRP and serum iron values. Normal frequency distribution test (Kolmogorov-Smirnov test) and CRP correlations with other parameters (Spearman correlation coefficient) are shown in Table 2.

Statistically significant differences (P<0.05) for CRP, Fe, TIBC, WBC and MCH values indicate deviation from the normal data distribution of the analyzed parameters (KS test). Between CRP and almost all parameters (except WBC and UIBC) a negative correlations were found while significant differences were determined for the WBC and MCV (P<0.05), Fe, Hb, HCT, MCH and MCHC (P<0.01).The correlation of serum iron and CRP values are presented in Figure 1.

Figure 1. Concentration and correlation of CRP and serum iron (Fe) in preschool children.



The highest value of CRP fits the lowest concentration of serum iron and vice versa. Figure 2 and Figure 3 show the normal probability plot for CRP and serum iron (Fe).

Based on Q-Q plot it can be observed that most of the results for both parameters were not normally distributed and also deviation of CRP is much higher in comparison to serum iron.

Figure 2. Probability or Q-Q plot of Serum Iron (Fe)

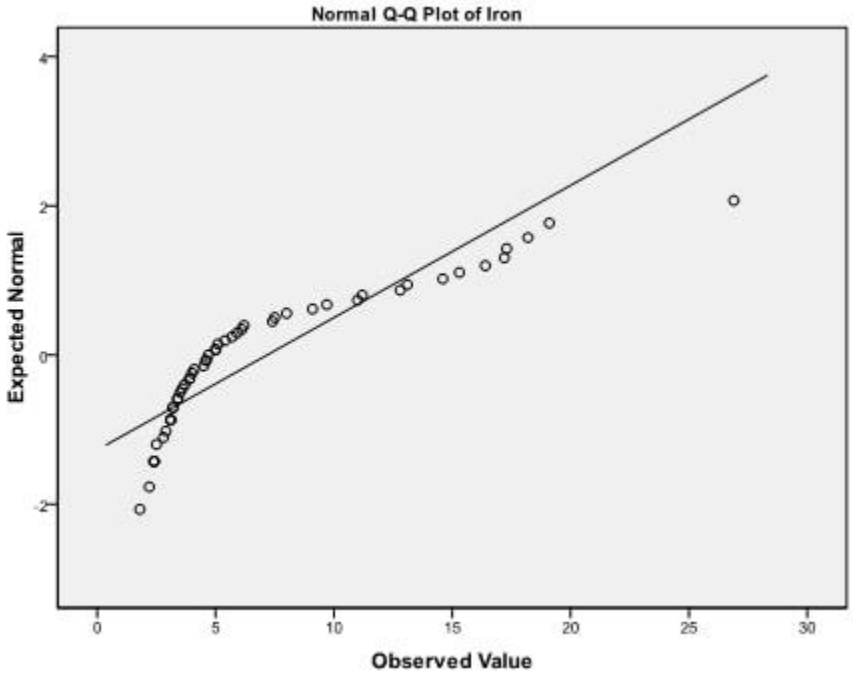
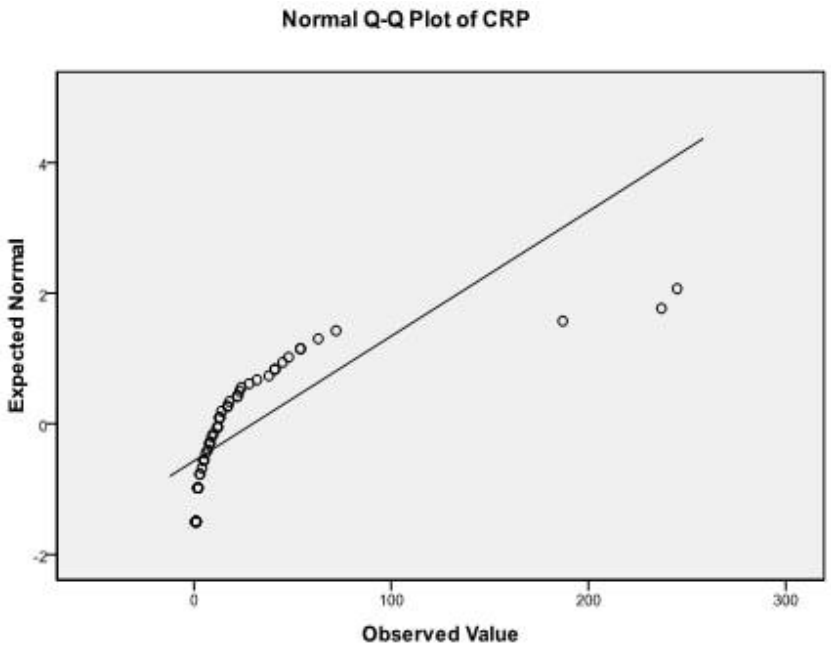


Figure 3. Probability or Q-Q plot of C-reactive protein (CRP)



DISCUSSION

CRP is present in low concentrations (<5 mg/L) in healthy individuals. In the course of anti-inflammatory response the concentration of CRP rises very fast, reaching its maximum in two days. CRP concentrations exceeding 100 mg/L are certainly linked with serious bacterial infections and autoinflammatory diseases (15, 16,17,18). A special aspect of our study was focused on analysis of serum iron whose values were elevated during acute inflammatory processes in children. Values of serum iron in children in our study were lower in comparison to other studies (19,20,21,22). Decreased serum iron levels were monitored and they were followed by decreased concentrations of TIBC and UIBC. The reason lies in the fact that during an inflammatory process hepcidin synthesis increases and it incorporates into the cell membranes involved in iron metabolism. The process of synthesis and membrane incorporation of hepcidin, prompted by IL-6, causes the iron to be trapped mainly in the enterocytes, macrophages and cells of the liver. Low serum iron values are considered to be the body's defending mechanism against infection, because the reduction of iron also reduces the available iron that microorganisms use in many metabolic pathways (23, 24). Soluble transferrin receptor (sTfR) is generally unaffected by inflammatory status, whereas ferritin increases along with acute-phase response in children (25). A mutation of NLRC4 gene can lead to a serious inflammatory flare that is similar to, or a form of, the macrophage activation syndrome (MAS). In such cases the biochemical analysis during MAS-like flares included elevation of C-reactive protein in serum and an extreme hyperferritinemia (26). White blood cell count is an important parameter during infection, although some previous studies have not shown significant changes of this value (27). In our study the number of leukocytes also did not deviate from the reference value, although patients with an inflammatory process often have an increased number of leukocytes (7). No correlation between the level of leukocytes and CRP values was observed in our study. Weak leukopoiesis response and little or no increase in the number of leukocytes in children is most likely a result of the time necessary for their mobilization, activation and adequate immuno-modulating chemotaxis in peripheral circulation, which is very short in the acute process. Hemoglobin concentrations were within reference ranges. According to the localization of hemoglobin in erythrocytes and due to the fact that it is not a participant in the anti-inflammatory response, changes in hemoglobin concentrations were not observed during acute inflammatory processes. Hematocrit values were within the reference range (7). Red blood cells parameters (MCV, MCH and MCHC) were within reference ranges, as confirmed by previous studies carried out during acute inflammatory conditions (7,22).

However, during chronic inflammatory processes the changes of these parameters were

evident as a result of compensatory response of hematopoietic centers (28,29,30,31,32). No statistically significant differences were observed between genders. However, higher CRP and iron values and lower concentrations of UIBC and TIBC were observed in females. These results are confirmed by previous studies (19,20). Lower iron concentration and higher TIBC and UIBC concentrations in males compared to females indicate that iron concentration is an interesting diagnostic marker in the inflammatory process. Hemoglobin concentrations were higher in males, which is a result of increase of the total amount of iron (Fe + UIBC). High concentrations of CRP correlated with a decreased serum iron observed in our study (2), which is considered a result of intensive and invasive immune responses. The ratio of CRP to serum iron shows that the concentration of serum iron directly depends on CRP values. It is evident that the patients with maximum CRP values have the lowest concentration of serum iron and vice versa. Amounts of free iron are only available during inflammatory processes. Spearman's coefficient of variation showed a statistically significant correlation between serum iron and CRP ($P<0.01$). CRP and cytokines affect the redistribution of iron in the liver and the mononuclear phagocyte system. As a consequence, a reduction of iron-binding proteins in plasma occurs. Decreased serum iron prevents its use by microorganisms and prevents the emergence of pro-oxidation potential of iron, which often leads to tissue damage due to the creation of ROS (33). Furthermore, inadequate values of hs-CRP were associated with severe obesity and high systolic blood pressure (35). These markers can be used to identify children and adolescents with higher risk of developing atherosclerosis later in life. Another study (35) concluded that obese children and adolescents have significantly increased hs-CRP compared with a normal weight group. However, the limitations of this study were the absence of a control group and an insufficient number of patients, so further research should be conducted on this topic.

CONCLUSIONS

Significant increase of CRP, slight increase of leukocytes and a decrease of serum iron during an inflammatory process in children were evident in our study. Values of TIBC and UIBC did not show diagnostic importance. A higher number of leukocytes in chronic response is expected. Increased CRP associated with lower serum iron levels might be considered as a biomarker of inflammatory processes in children. Furthermore, TIBC and UIBC together with serum iron levels might be prognostic markers of anemia due to chronic inflammation status. However, these findings need to be confirmed by large prospective cohort studies.

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Shear Wave Sonoelastography as an Important Diagnostic Tool in the Diagnosis of Breast Focal Lesion with Unclear Ultrasound Features

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Abstract

Aim: The evaluation of the role of Shear Wave Sonoelastography (SWE) in the diagnosis of the lesions with unclear ultrasound features.
Materials and method: 139 solitary lesions were evaluated in 2178 female patients examined in 2015. The age span was 20-70 years old. 34 cases selected had unclear ultrasound features. The patients underwent SWE and afterwards biopsy. The patients with clear ultrasound features for malignant or benign lesions and the patients that the last trimester underwent therapeutic or diagnostic invasive procedures were excluded from this study. The data concerning malignant lesions are presented in Table 1 and the data concerning benign lesions are presented in Table 2. The mean value of stiffness in malignant lesions has been compared with the one of benign lesions and the mean value of stiffness in fibroadenomas has been compared with the one in fibrocystic mastopathy. Statistical analysis was performed utilizing the student's test (t test) according to SPSS package (version 19.0 for Windows, SPSS Inc.,Chicago, Illinois, USA).
Results: 6 were malignant lesions, while 28 were benign. The differences between the averages of the stiffness values of malignant lesions and benign lesions (fibroadenoma and fibrocystic mastopathy) were statistically important according to student's test ($p<0,0001$). For the benign lesions, these differences were not important ($p=0,7257$).
Conclusion: SWE is a valuable tool in the differentiation of breast malignant lesions with unclear ultrasound features, making ultrasound a sensitive modality in general.

Keywords: breast, ultrasound, biopsy, wave.

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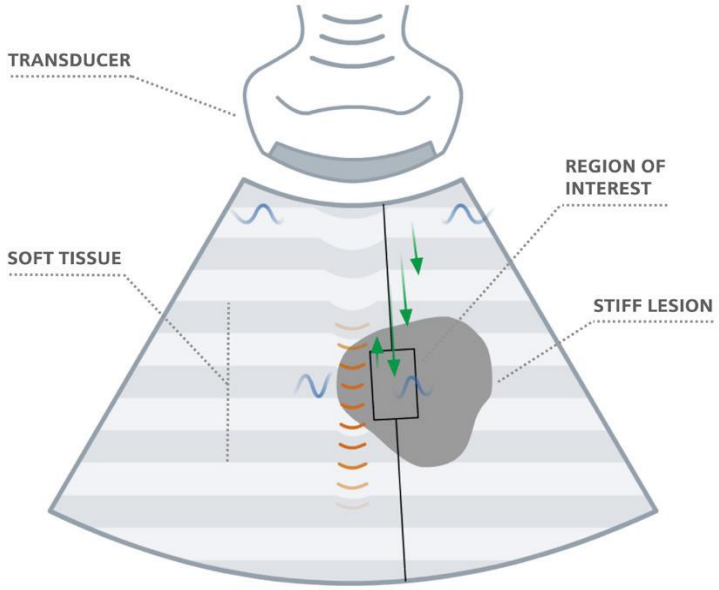
INTRODUCTION

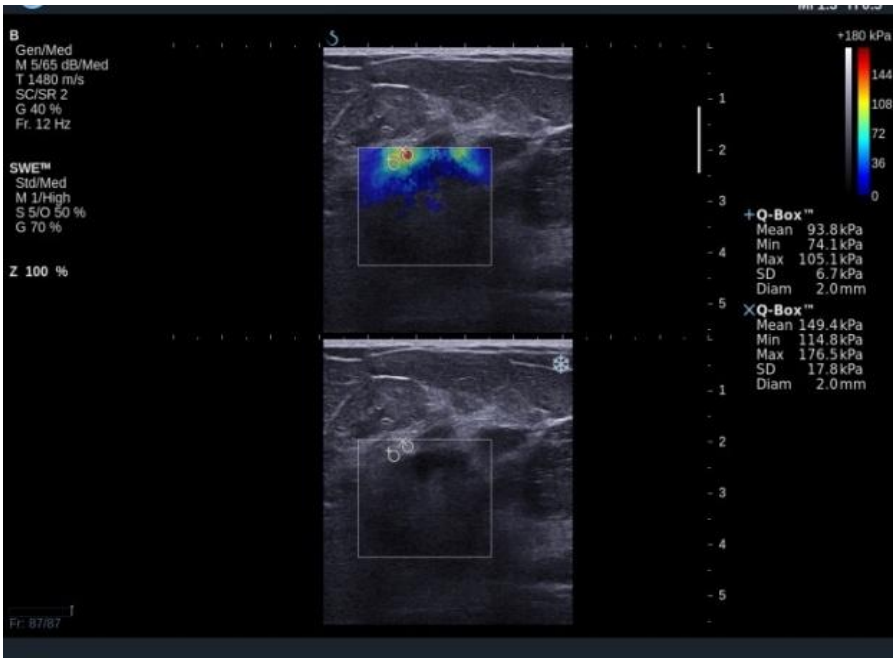
Actually, B-mode ultrasound combined with color Doppler is considered as the most important modality in routine breast exams, especially in the case of breasts with dense parenchyma and Bi-RADS 3 and 4 in mammography. Recent studies claim that ultrasound has some problems, especially regarding the over-diagnosis of unclear lesions and hence the recommendation of unnecessary biopsies (1). According to an author "Supplemental ultrasound screening for women with dense breasts has a high false-positive rate and substantially increases the number of unnecessary biopsies with little gain in quality-adjusted life years (QALYs)" (2). According to some other authors, the use of the ultrasound as a complementary method is not so cost-effective as "Supplemental ultrasonography screening for women with dense breasts would substantially increase costs while producing relatively small benefits" (3). To avoid these problems nowadays 3D mammography is being introduced in the practice of breast imaging exams, known also as digital breast tomosynthesis (DBT). According to some authors, the implementation of DBT has increased with 41% the chance of detecting invasive cancers. Regarding in situ carcinomas (DCIS) there is no change while it has decreased the unnecessary examinations for false-positive cases (4). However, it is still impossible to have DBT in all the centers for breast exams. Showing a consideration for the conclusions of the above mentioned authors, we can say that technological advances in ultrasound techniques tend to surpass these handicaps. One of these new methods is Shear Wave Sonoelastography (SWE). In conformity with some publications, SWE is an important tool, which is being developed to differentiate breast lesions and to decrease the number of unnecessary biopsies (5, 6, 7). This hypothesis is also supported by this study. Even if the physical principles of SWE don't represent the subject of this study, it is necessary to present a brief explanation of this technique. This modality uses automatic compressing pulses generated by the ultrasound probe which causes compression within the tissue under examination. This compression induces transversely oriented shear-waves. The speed of propagation of the shear-waves can be captured by the ultrasound system with the same probe. This speed is directly proportional to the stiffness of tissue, that means stiffer the tissue, higher the speed of propagation of shear-waves. The reconstruction software of the machine uses the formula $E \approx 3\ \rho v^2$ and Young's modulus to produce the electrographic image and to calculate the elasticity in kPa. Using extremely fast ultrasound acquisition sequences of 5000 frames/sec, the shear-waves and an associated elastogram can be subsequently captured in real-time (8). (Figure 1).

Figure 1. In this scheme it is shown how are produced and work shear –waves.



The color elastogram ranges from dark blue for the softest tissue to deep red for the hardest one. The values of the elasticity are measured placing the ROI circle inside the elastogram box (Figure 2c, d-3c).

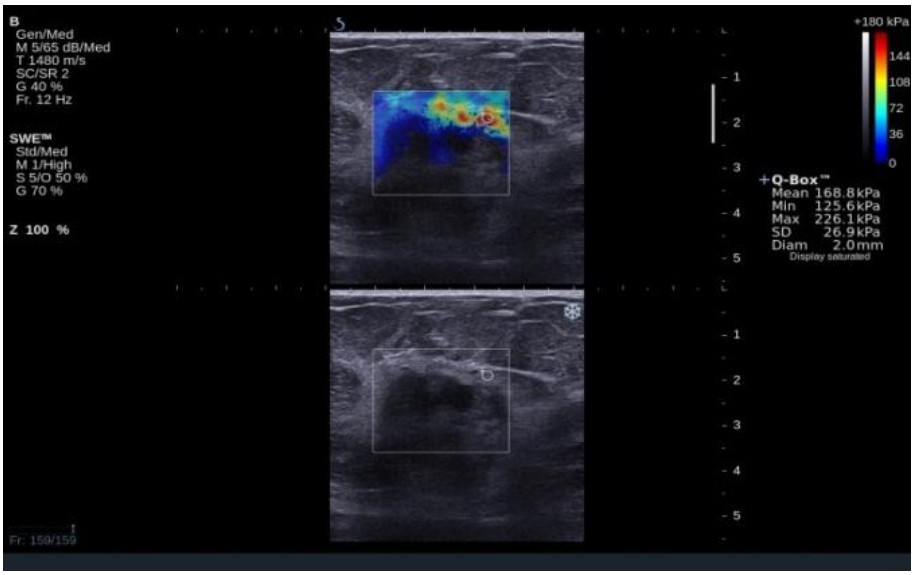
Figure 2. a-d. US images in B-mode shows an unclear lesion (white arrows) in the medial inferior quadrant of the right breast. The lesion was not evidenced in two previous ultrasound exams within the last trimester (a, b).



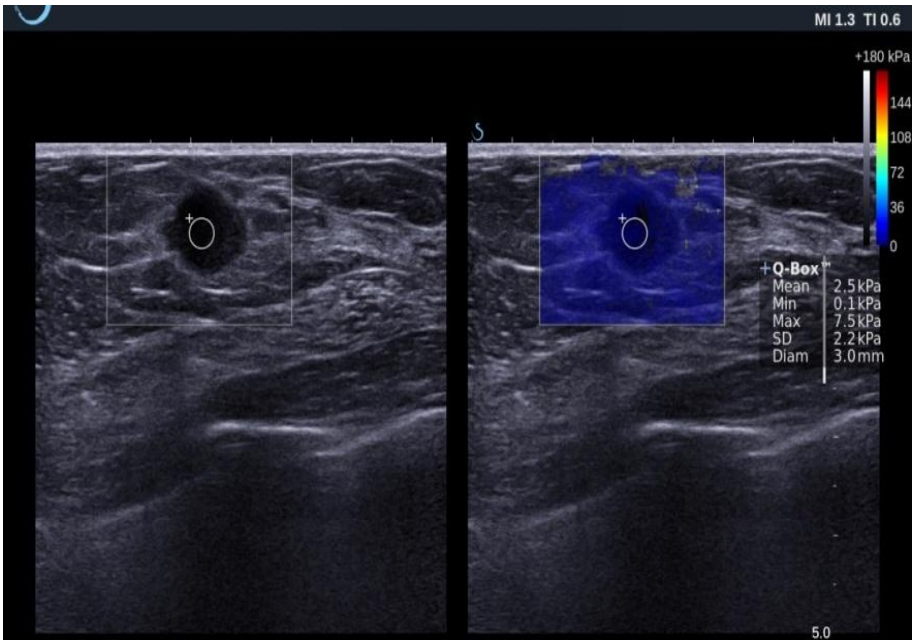
2 c

SWE shows stiffness up to 149 kPa in the tissue around the lesion, which is suspicious for malignancy BI-RADS 4. Yellow to red colors show the hard peripheral desmoplastic rim of the lesion. Blue color shows surrounding normal breast tissue (c, d). Core biopsy was performed and it resulted IDC grade II.

Figure 3. a-c. Hypoechogeneous lesion with clear polygonal contours (arrows) and diameters almost equal(a). There is minimal vascularisation (arrow) in the periphery (b). In SWE the values are low, approximately 2.5 kPa which suspects a benign lesion, that resulted fibroadenoma in biopsy. All the field inside the box is blue that means low stiffness (c).



2d

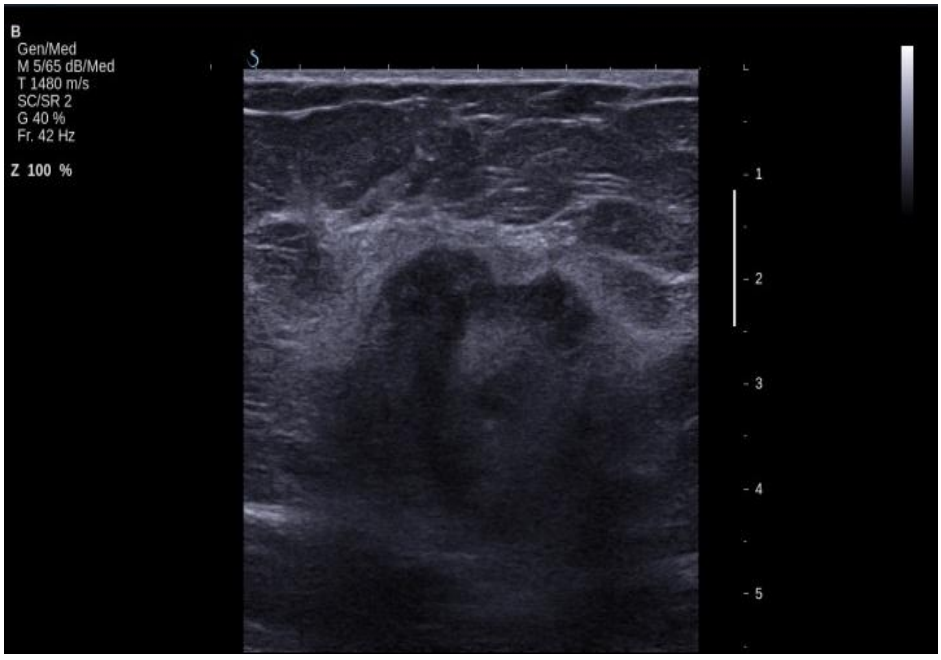


3c

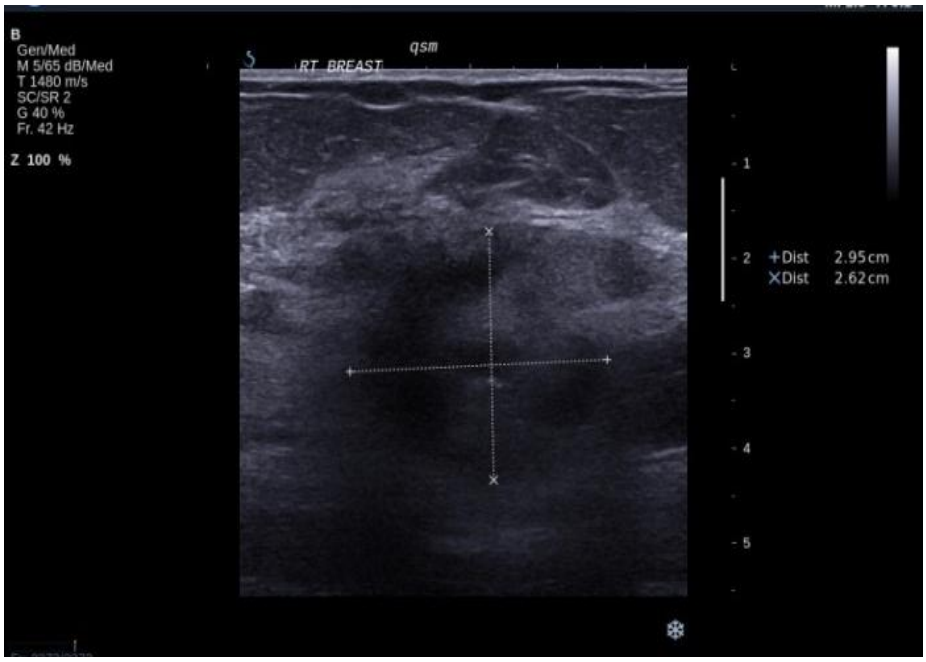
MATERIAL AND METHODS

This is a retrospective study that has reviewed 139 cases with solitary lesions in 2178 women examined during 2015. The age span was from 20 up to 70 years.

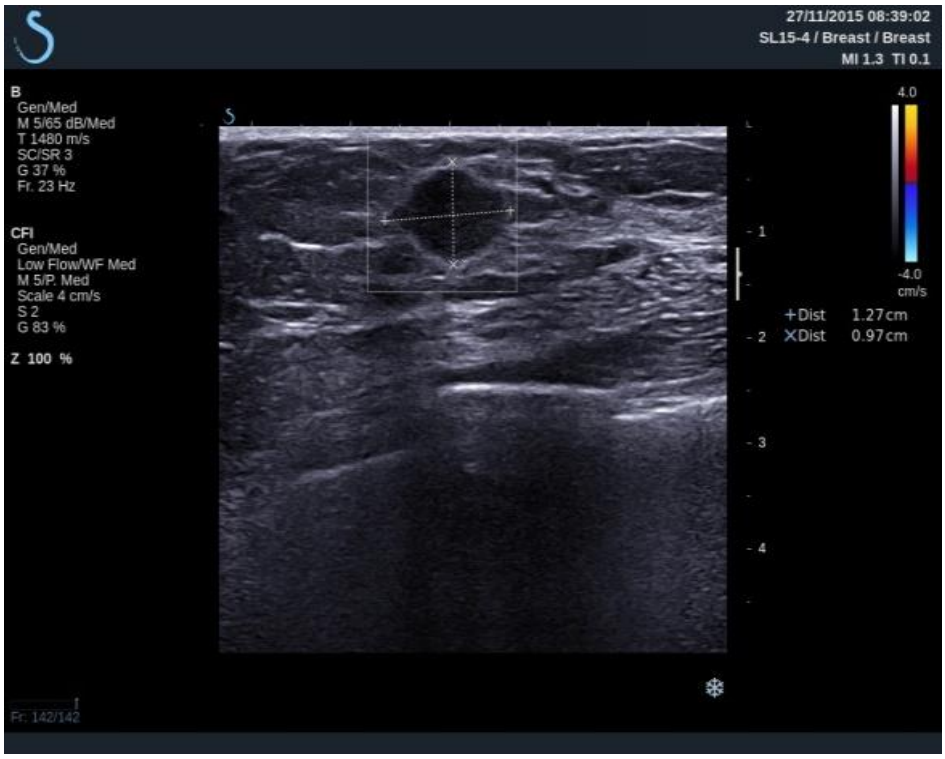
At the time of completion of this study all the diagnostic procedures of the above mentioned patients had finished. The case files were chosen according to the following criteria: 34 patients with lesions with unclear ultrasound features were selected. (Figure. 2a,b-3a,b)



2a



2b



3a

The patients with clear ultrasound features for malignant or benign lesions, as well as the patients who in the last 3 months underwent invasive therapeutic or diagnostic procedures were excluded from this study. The patients underwent B-mode US, SWE and subsequently biopsy exams. These exams were performed by professionals with 10 year experience in ultrasound exams and breast biopsies, and with at least three year of experience in utilizing the SWE method. Every sequence of SWE was at least 10s and minimally two orthogonal sequences were taken for every lesion (Fig.2c-d). ROI diameter was 2 mm. The threshold value for considering a lesion probably benign was taken 50kpa[5].

Higher values lesions were suspected as malignant. We find appropriate to explain this is a standard exam protocol in our cabinet. Afterwards, we did the correlation between the biopsy results and the SWE values.

Statistical analysis was performed utilizing the student's test (t test) according to SPSS package (version 19.0 for Windows, SPSS Inc., Chicago, Illinois, USA).

The mean value of stiffness in malignant lesions was compared with the one of benign lesions, and the mean value of stiffness in fibroadenomas was compared with the one in fibrocystic mastopathy. The SWE exams were performed with ***SuperSonic Imagine Aixplorer*** (Provence, France) device with linear probe SL15-4.

Tru-Cut biopsies and FNA-s were performed with ***GE LOGIQ S7*** expert device (GE Healthcare, United Kingdom) with linear probe 11L. The material was taken utilizing 16G or 18G needles, with 10-20 mm cut.

DISCUSSION

As it was proven previously, SWE improves the ability of the traditional ultrasound to identify the malignant lesions even when they have no clear characteristics.

Data Malignant Lesions	Stiffness (mean value)	Grade of malignancy	Age
IDC	149	2	46
IDC	170	3	55
IDC	110,5	2	61
IDC	137,8	3	72
Mucionous carcionoma	72	1	39
Mixt type	81	1	44
Mean value	120		

IDC (infiltrative ductal carcinoma).

Table 1. Values of stiffness and histological correlations for malignant lesions.

RESULTS

The data are displayed in Table 1 for malignant lesions and in table 2 for benign lesions.

6 cases out of 34 were malignant lesions, while 28 other were benign. The differences between the averages of the stiffness values of malignant lesions and benign lesions (fibroadenoma and fibrocystic mastopathy) were statistically important according to student's test ($p<0,0001$). For the benign lesions, these differences were not important ($p=0,7257$).

This is confirmed also by the results from some other publications that has similar conclusions with us, like Berg WA et al.(8), which shows improvement of specificity from 61,1% to 78,5% by using color and from 69,4% to 77,4% using elasticity values in kPa, without significant improvement in sensitivity. Also Au FW and Ghai S cite "there was a statistically significant difference in the values of the quantitative shear wave elastography parameters of benign and malignant solid breast masses",

and further "by adding shear wave elastography parameters to BI-RADS category 4a masses, we found that about 90% of them could be correctly downgraded to BI-RADS category 3, thereby avoiding biopsy". Another group of authors (Lee SH et al) (10), reports increased specificity from 17,4% to 62,1% for SWE color stiffness and 53,3% for elasticity values without loss in sensitivity.

The same authors have concluded that "The addition of SWE to B-mode US improved diagnostic performance with increased specificity for screening US-detected breast masses. BI-RADS category 4a masses detected at US screening that showed a dark blue color or a maximum elasticity value of 30 kPa or less on SWE images can be safely followed up instead of performing biopsy."

Mean value stiffness Fibradenomas	**Age**	**Mean value stiffness Fibrocystic m.**	**Age**
21	17	27,5	27
18	22	23,2	35
19,5	27	33	21
29	32	47	57
25	17	36,5	41
23	52	67	48
21	46	31,7	36
41	24	18	19
2,5	37	16	29
18	33	55	38
31	26	25	50
Mean value 22,6	30,33	24	42
		53	26
		14,8	33
		22	43
		21,7	22
		Mean value 32,2	36

Table 2. Values of stiffness and histological correlations for benign lesions.

There is a lot of evidence supporting the advantages of SWE as a novel method in breast masses diagnosis, but we cannot forget that even SWE has its limitations, as: it is an examination that lasts and is more expensive than B-mode US. The interpretation is depended on the imaging specialist experience, besides the improvements done to the SWE technique. Despite of the improvements regarding the ultrasound sensitivity and specificity, SWE is still behind in comparison to the tru-cut biopsy ultrasound guided, which has 91% sensitivity and 98% specificity (11).

As previously mentioned, with this innovative method, it is possible to reduce the unnecessary cases recommended for tru-cut biopsy, especially them with unclear ultrasound features, but not to minimize totally.

CONCLUSION

SWE is a valuable tool in the differentiation of breast malignant lesions with unclear ultrasound features, making ultrasound more sensitive modality in general. This method is not valuable to discriminate the benign lesions between them.

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Conflict of interest disclosure: Not available.

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A 16 Year-Old Girl with Fever, Abdominal Pain and Progressive Neurological Deficits

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Abstract

Introduction: Listeria monocytogenes is a small gram positive bacillus that can be isolated from soil, vegetation or animal reservoirs. Human disease occurs mainly in immunocompromised people, neonates and in pregnancy while the cases in immunocompetent people are rare. CNS manifestations of the disease can be in form of meningitis, encephalitis and also cerebritis since L. monocytogenes shows tropism for brain and brain stem as well for the meninges.

Case presentation: A 16 year-old girl, who had been previously healthy, came at our attention because of progressive neurological deficits. The symptoms of the disease were present two months before the admission when she had experienced intermittent and severe headaches and fever up to 38 C, nausea, vomiting and abdominal pain lasting for 5 days. Her symptoms included lethargy and pain at the moment of admission.

After having developed diplopia and unsteady gait after three weeks she was admitted in our department. Multiple cranial neuropathies were present. Neurological diagnosis was rhombencephalitis. CSF analysis showed a colorless fluid, 10 lymphocytes/mm3, 77 mg/dl protein, and 69 mg/dl glucose.

Serum samples obtained during the admission were positive for Listeria and she was treated with ampicillin and gentamicine accordingly, with a good immediate response.

Conclusion: The most important feature of this paper is its extended differential diagnosis comprising tumoral, inflammatory, vascuar and infectious diseases. Thus, it represents a valuable aid for all concerned specialist of above-mentioned fields.

Keywords: pontine lesion, rhombencephalitis, MRI, Listeria monocytogenes.

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CASE PRESENTATION

A16 year-old girl, in previously excellent health status, resident in the rural periphery of Prishtina (Kosova) was admitted at our department because of progressive neurological deficits.

Approximately 2 months before this admission, she began to have intermittent and severe headaches and fever up to 38 C, nausea, vomiting and abdominal pain lasting for 5 days. She was admitted at that time to the emergency service of the local hospital.

During admission at the above mentioned hospital her symptoms included lethargy and pain. Her temperature was 37.9°C, and her pulse 112 beats per minute; respirations were 20 breaths per minute, and the oxygen saturation was 95% while the patient was breathing ambient air. Her pupils were equal and reactive to light and accommodation. Mucous membranes were dry and pink. The neck was moderately rigid.

Question for consideration: Discuss on the principal syndromes towards this clinical picture.

COMPLEMENTARY EXAMS

Numerous causes, ranging from acute life-threatening emergencies to chronic functional disease and disorders of several organ systems can generate abdominal pain, fever and suddenly headaches. Among the most common causes, the following can be listed:

- gastro-enteritis (inflammatory disease, bacterial or viral infection, obstruction)
- meningitis (nuchal rigidity, headache, photophobia, and prostration; may not be febrile)
- intracranial especially subarachnoidal hemorrhage (nuchal rigidity and headache; may not induce clouded consciousness or seizures. Hemorrhage may not be seen on CT scan. Lumbar puncture shows “bloody tap” that does not clear by the last tube.

Complete neurological exam is an essential first step. If this exam is abnormal or if it is serious underlying cause is suspected for any reason and as a result an imaging study (CT or MRI) is indicated.

A computed tomographic (CT) scanning of brain was performed showing no abnormality. The cerebrospinal fluid (CSF) was non-hemorrhagic. Biochemical exam showed three red blood cells/mm3, 70 white blood cells/mm3 (63% neutrophils and 37% mononuclear cells), a protein and glucose level of 40 mg/dl and 63 mg/dl respectively. Results of routine hematologic tests; tests of coagulation, renal function, and liver function; the level of C-reactive protein; electrolyte levels; and a toxicology screen were normal.

Since the abdominal pain and vomiting persisted, a fibrogastroscopy was performed; The final diagnosis was given:‘acute gastrointestinal infection’ and a symptomatic treatment was prescribed.

Three weeks after, the patient developed diplopia and unsteady gait and she was admitted at our department. Multiple cranial neuropathies were present: bilateral internuclearopthalmo-plegia, horizontal and vertical nystagmus, right trigeminal palsy (right hemi-facial hypoesthesia, direct and consensual corneal hyporeflexia, oblique mouth) right peripheral facial palsy (grade III according House Brachman), mixed nerve palsy (fausse-routes), vertigo and static and dynamic ataxia.

Question for consideration: From semiology to topography, where it may be the lesion location?

NEUROLOGICAL REASONING AND TREATMENT

A brainstem syndrome is clinically suspected when the patient presents diplopia, ataxia, disturbance of consciousness. By means of the Rostro-caudal plan, the lesions are categorized as:

1) **Rostral medulla**: massive bulging of the dorso-lateral area due to the restiform body;

2) **Middle medulla**: bulging of the lateral surface due to the inferior olive;

3) **Caudal medulla**: relatively round shape without bulging of the lateral surface.

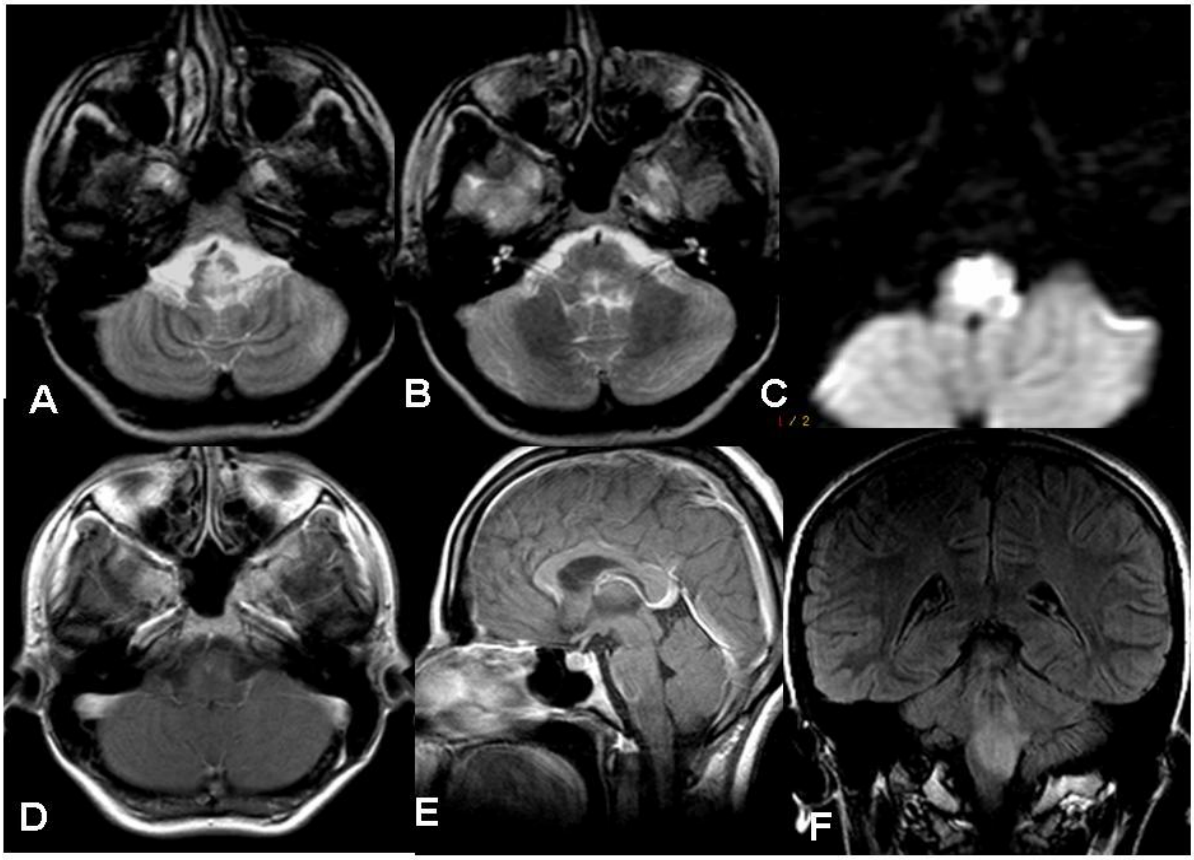
Clinically, the patients of the rostral group usually present dysphagia, dysarthria, facial paresis and a bilateral trigeminal sensory pattern significantly more often severe than caudal group patients; whereas gait ataxia, headache, isolated limb/body sensory pattern and sensory gradient worse in the leg than in the arm are significantly less often than caudal group patients.

Using horizontally axes, the classification is as follows: 1) **Typical type**: Diagonal band-shaped lesions sparing the most dorso-lateral portion, most common; 2) **Ventral type**: Similarly shaped, but more ventrally situated lesions involving some portion of the inferior olive and sparing relatively large portions of the dorso-lateral area; 3) **Large type**: Large lesions extending ventrally so as to involve some portion of the olivary nucleus and dorsally to involve most of the dorso-lateral area; 4) **Dorsal type**: Lesions restricted to the most dorsal or dorso-lateral portion; 5) **Lateral type**: Some lesions were restricted to the lateral, superficial area without extending dorsally; 6) **Unclassifiable type**: Other lesions not classifiable.

Clinically, the frequencies of dysphagia, dysarthria, Horner sign and bilateral trigeminal sensory pattern are significantly different among horizontal subtypes: ‘large type’ lesions tended to have frequent dysphagia, hoarseness, dysarthria and bilateral trigeminal sensory pattern. These symptoms were uncommon in those with lateral lesions. Horner sign tended to be uncommon in pts with dorsal lesions.

Cerebral magnetic resonance imaging (MRI) showed an increased T2-weighted signal associated with restricted diffusion involving the brainstem, the left middle cerebellar peduncle and cervico-medullary junction which was interpreted as a demyelization process (Fig. 1).

Fig. 1. Cerebral MRI at admission. Hypersignal in cervico-medullary junction at axial T2-weighted (A and B) as well as Flair (F) with homogeneous restriction in DWI (C) and scant contrast enhancement in axial (D) and sagital (E) scans.



Subsequently, the patient received a 3-day treatment of Methylprednisolone sodium succinate (Solu-Medrol) (1g/day). Since the clinical status of our patient deteriorated, thus she was admitted at our department.

Questions for consideration:

- discuss on the radiological differential diagnosis of this lesion.
- which complementary diagnostic test would you recommend?

DISCUSSION AND FINAL DIAGNOSIS

The radiological findings at this case are compatible with rhombencephalitis.

Rhombencephalitis is serious, uncommon illness originally described by Bickerstaff and Cloake (1), which is very difficult to diagnose clinically. Patients typically present with symptoms of areflexia, ataxia, and ophthalmoplegia (1).

The etiology is frequently undetermined; there are many inflammatory, vascular, neoplastic, metabolic and demyelination conditions which

show radiological features similar to those of our patient on magnetic resonance imaging (MRI).

Acomplete and exhaustive laboratory work-up was performed: white-cell count of 26,200 cells per mm3 (84.8%-neutrophils), eritrose-dimentation rate of 30mm/h, C-reactive protein of 4mg/dl. The rest of tests (renal and liver function, electrolytes) was normal. Routine cerebrospinal fluid (CSF) exam showed a colorless fluid, 10 lymphocytes/mm3, 77 mg/dl protein, and 69 mg/dl glucose. Cultures of the cerebrospinal fluid and blood testing for infectious agents (including cytomegalovirus, Epstein–Barr virus, herpes simplex virus [HSV] type 1 and type 2 and for Lyme, lysteria monocytogenes and syphilis were negative. Tests for angiotensine-converting enzyme and antinuclear antibodies were negative on blood and CSF. CSF PCR for Whipple disease was negative as well.

Cerebrovascular Disease

The progressive development of neurological disorders and the young age of our patient make this diagnosis unlikely. In this patient, the results of magnetic resonance angiography ruled out vascular occlusion and the cerebrospinal fluid findings suggested causes other than stroke.

Central Pontine Myelinolysis (CPM)

It constitutes the major risk of developing cerebral demyelinating lesions as a result of a rapid correction of a chronic hyponatremia. Given the fact that our patient did not present hyponatremia and the MRI lesion lacked the characteristics of CPM (triangular lesion located ventrally in the basis of pontis with sparing of tegmentum and corticobulbar tracts), this diagnosis seemed less probable.

Demyelization Disorders

The patient was treated with corticosteroids, probably because a demyelinating disorder was considered. However, this 16-year-old girl had no history of visual or neurologic deficits. The findings of MRI lacked the characteristics of demyelinating process. The absence of oligoclonal band on CSF and of a clinical response to corticosteroids does not also support the diagnosis of multiple sclerosis.

Neuro-sarcoidosis

Sarcoidosis can affect the CNS with basal meningitis and cranial neuropathies. Parenchymal lesions preferentially occur in the brain stem and hypothalamus (2). However, the symptoms of sarcoidosis are not as acute as those in this patient and the cranial nerves, The normal level of angiotensin-converting enzyme in the cerebrospinal fluid and the absence of pulmonary manifestations argued against the diagnosis of sarcoidosis (3).

CNS Whipple disease (WD)

Among the systemic manifestations of WD, our patient presented with abdominal pain and fever and lacked chronic migratory arthralgias or polyarthralgias (which for several years precede the onset of neurological symptoms) and unexplained weight loss. However, 4-6% of patients with CNS-WD had no systemic symptoms or signs (4). The absence of PCR in CSF makes inconclusive this diagnosis.

Inflammatory Diseases

Systemic lupus erythematosus (SLE) commonly has systemic manifestations, including the skin, joints and kidneys (5).

Neurologic manifestations include psychiatric or behavioral changes, seizures, and peripheral neuropathy; however, the clinical picture and the negative antibody testing and the oral and genital ulcers in this case rule out a diagnosis of SLE. Basal meningitis can occur in Wegener's granulomatosis, causing cranial neuropathies, headaches, strokes and seizures. The cerebrospinal fluid profile shows a mild-to-moderate lymphocytic pleocytosis and increased total protein and IgG index. The antineutrophil cytoplasmic antibodies in our case was negative, as well as the lack of pulmonary, sinus, or renal involvement, Wegener's granulomatosis was unlikely (6).

Neuro-Behçet's Disease

In addition to oral and genital ulcers, uveitis, skin lesions, arthritis, thrombophlebitis, a positive family history, and gastrointestinal, CNS, or vascular involvement are common features of the disease (7, 8). The absence of any of these manifestations as well as lack of improvement after corticotherapy ruled out this hypothesis.

Infectious Diseases

HSV encephalitis has a predilection for the temporal lobes and may be necrotizing and hemorrhagic (9). The most important, PCR testing for HSV DNA, a highly sensitive and specific assay was negative, making this diagnosis highly unlikely.

Meningovascular syphilis may occur within the first 12 months after primary infection and is associated with headache and meningismus (see Figs 6 and 8, page 257 of Greenfield's Neuropathology (10). It produces a basal meningitis, and cranial-nerve palsies (seventh, sixth, or second cranial nerve, in decreasing order of occurrence) are common (10). The absence of genital ulcers and the negativity of testing for syphilis make less likely this disease

Other less likely infectious possibilities include: t*uberculosis, varicella–zoster virus infection, and Lyme disease*. Serologic testing for Lyme disease and Varicella–zoster virus were negative. *Listeria monocytogenes* is an uncommon etiologic agent of infection in general population. However, being an important cause of severe infections in neonates, pregnant women, the elderly and other individuals with impaired cell-mediated immunity (11). Several clinical syndromes related to Listeria infection have been described, like gastroenterocolitis, sepsis, endocarditis, central nervous system (CNS) infections, etc. CNS disease caused by Listeria includes meningitis, diffuse encephalitis and well-localized abscesses (11).

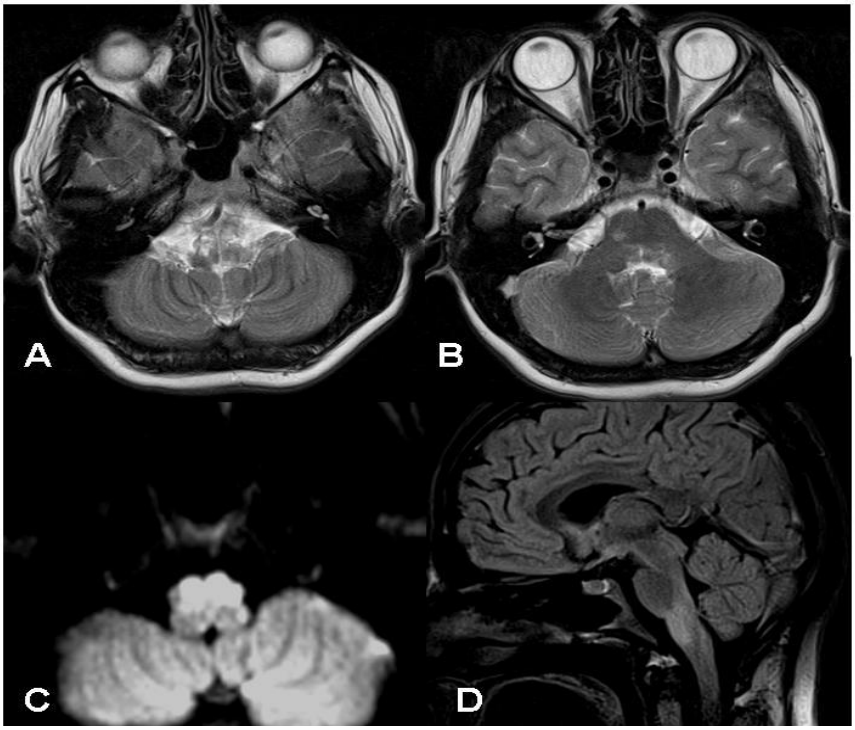
Listeria rhombencephalitis is a rare and potentially life-threatening infection. The brainstem predilection mechanism remains unexplained adequately.

Majority of cases reported are sporadic, occurring in previously healthy individuals (12). The clinical course is usually biphasic, with unspecific symptoms consisting of headache, malaise, nausea, vomiting, fever in the first 4–10 days, followed by progressive brainstem dysfunction with cranial nerves palsy, cerebellar signs, hemi- or tetraparesis, sensory deficits, respiratory insufficiency, impairment of consciousness and sometimes seizures. Initial CT of the brain usually remains normal and the MRI shows hyperintense, patchy lesions within the brainstem and/or multiple microabscesses (12). However, isolated midbrain localization was rare (3%). Meningeal signs were present in 48% of cases.

Typical markers of inflammatory response, like elevation of CRP and leucocytosis, increased percentage of immature granulocytes are usually absent. This can be explained by mainly intracellular spread of Listeria (13).

Interestingly, CSF often remains sterile with only mild, unspecific abnormalities (14). Low-grade pleocytosis (mean, 392 cells/mm3), usually with lymphocytic predominance, and low-level hyperproteinorrachia (mean, 99 mg/dL) occurred in 88% and 89% of cases, respectively, and hypoglycorrhachia occurred in 21% (1, 11, 14). Gram stains of CSF resulted positive in 14% of cases, as opposed to 28% in listerial meningitis and 60% in other bacterial meningitides.

Fig. 2. Cerebral MRI one after the onset of treatment revealing an attenuation of hypersignal in axial T2-weighted (A and B) and in sagital Flair (D) as well as diminuation of the zone with diffusion restriction (C).



CSF cultures were positive in 42% of cases, as opposed to 90% in listerial meningitis (15), most often late after admission.

Our patient's presentation at the age of 16 was more consistent with Behçet's disease; serum samples obtained on admission were positive for Listeria and she was treated with ampicillin and gentamicine accordingly with a good immediate response (Figure 2).

Successive MRI showed the quasi complete disappearance of the lesion (Figure 3).

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Specific author contribution at the study:

E.R. (concept, design, data collection, writing, reviewing, final approval)

A.R. (data collection, analysis, drafting, reviewing, final approval)

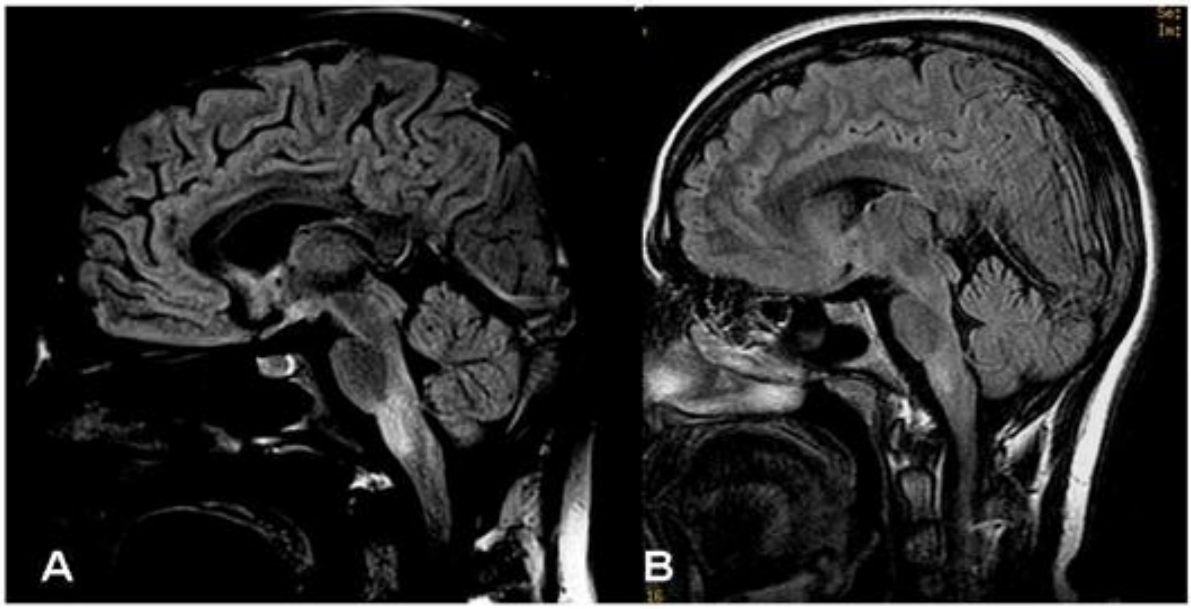

Figure 3. Net radiological response comparing the MRI of one month after the treatment (A) with that at 1 year of follow-up (B).

This case underscores that Listerial rhomboencephalitis is a syndrome observed predominantly in previously healthy adults without history of immunosuppression and emphasizes also the utility of a wide differential diagnosis.

M.P. (data analysis, differential diagnosis, drafting, reviewing, final approval)

G.K. (concept, design, data collection, writing, reviewing, final approval)

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A New Approach to the Pharmacological Treatment of Oral Lichen Planus: Case Report

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Abstract

Background: Lichen Planus (LP) is a chronic inflammatory mucocutanoeus disease with an unclear pathogenesis, in which the immune system plays an important role. In the majority of the cases the disease affects the oral mucosa, but it may also affect also the skin, the vaginal mucosa, the hair scalp and the nails. These lesions are generally multiple and almost always appear bilaterally with a symmetric distribution. In some cases the lesions represent erythematous and erosive characteristics mixed or superimposed on each other.

Case Report: The purpose of this article is to report a case of 79-year-old male with Oral Lichen Planus for more than 10 years. Previous therapies have proved partially success because their withdrawal was associated with a more aggressive reactivation of the lesions. Our aim is to report a new approach to the standard therapy for the management of the disease and the long term life improvement of the patients.

Conclusion: Improvement of symptoms in this case is achieved with the use of local corticosteroids. Since the treatments are not specific, they aim at eliminating the inflammation. Therefore, they are partially successful. Besides pharmacological treatment, maintaining oral hygiene and particularly the cooperation of the patient, are essential factors in the successful management of the disease.

Keywords: oral lichen planus, corticosteroids, long term therapy.

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INTRODUCTION

Lichen Planus (LP) is a chronic inflammatory mucocutanoeus disease with an unclear pathogenesis, in which the immune system plays an important role (1). In the majority of the cases the disease affects the oral mucosa but it may affect also the skin, the vaginal mucosa, the hair scalp and the nails (2,3). Its clinical forms are reticular, papular, plaque, erythematous (atrophic), erosive and bullous which is very rare. The unique characteristic is often presented under the appearance of lesions in parts of traumatized or damaged skin, also known as the *Koebner phenomenon.* In most cases, LP is limited to the oral mucosa. These lesions are generally multiple and almost always appear bilaterally with a symmetric distribution. In some cases the lesions represent erythematous and erosive characteristics mixed or superimposed on each other. Prevalence rates of oral lichen planus (OLP) vary from 0.5% to 2.2% of all population. The disease affects more women than men in a ratio 3:2. Most of the patients belong to the fifth decade with an average age of diagnosis 55 years (4).

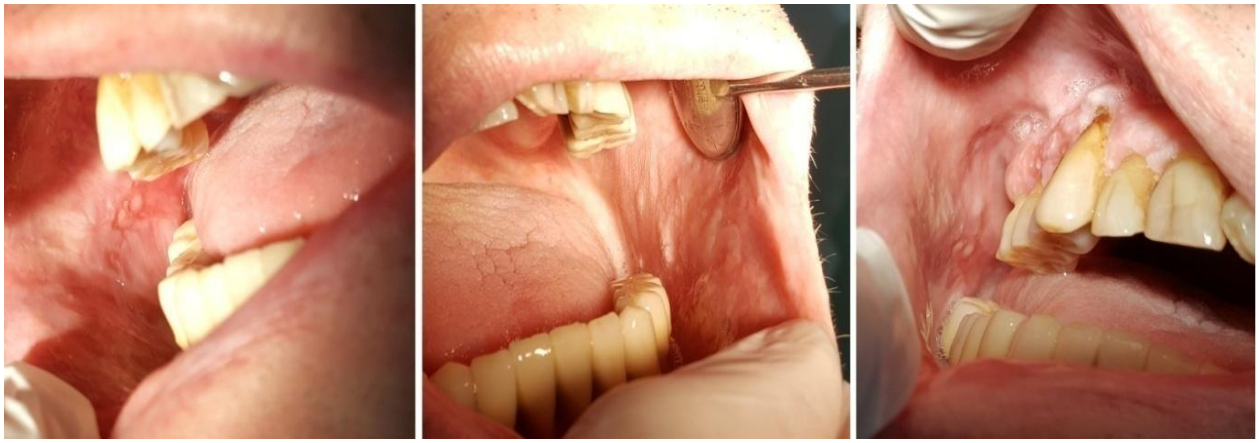
CASE REPORT

A 79 years old, male patient was referred to the University Dental Clinic of Tirana with the chief complaint of pain and burning sensation in the mouth during nutrition. The first symptoms appeared approximately 10 years ago. At that time, the patient was treated with clobetasol propionate 0.05%, 3times a day for 2 months. During that period, lesions showed improvement and no pain was present. After a period of interruption of the medication, a more aggressive reactivation of the lesions was noted associated with severe pain and burning symptoms during nutrition. During that period the patient did not use medications, instead he tried by himself to manage the symptoms changing his lifestyle and diet. Furthermore, after a few years the patient was treated by medical prescription with triamcinolone acetonide 0.5% cream 2 times a day for 1 month accompanied with topical gel for tissue regeneration with amino acids and hyaluronic acid. The therapy was partially successful. Four months after completion of the therapy, lesions appeared again and the patient complained as usually as in the past. . After 3 years from the last medical visit regarding this issue, the patient presented at our clinic. In a general examination, the patient reported surgery for cholecystectomy 37 years ago and he suffers from hypertension for more than 20 years which is perfectly managed by medications as stated. The subject uses digoxin, atenolol and aspirin. Recent blood tests showed values within normal range. No allergic reactions and no significant family history were reported. The patient does not smoke and rarely drink small amounts of alcohol. Tooth brushing is performed once a day with a horizontal movement without exercising force. Depression, anxiety and stress test (DASS) resulted negative. In a general perspective, the patient looked healthy.

A clinical intraoral examination showed bilateral erosive buccal lesions, in the right retro molar triangle and in the first quadrant extended from the second incisive to the first molar gingiva (Fig.1).

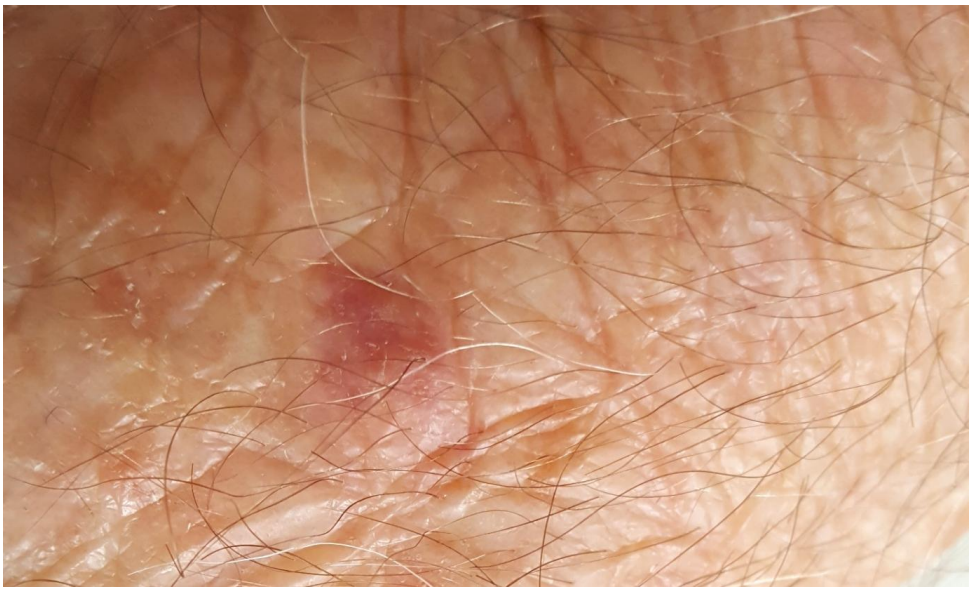
The preliminary clinical diagnosis was oral lichen planus. Differential diagnosis may include pemphigus, pemphigoid, lichenoid lesions associated with contact with restorative materials, leukoplakia and lupus erythematosus.

Fig. 1. Lesion distribution in the oral cavity at the first visit.



Extra oral examination resulted with purplish-colored lesions in the dorsum of the hands and a few nodules in the wrists (Fig.2).

Fig.2. Purplish-colored lesions in the dorsum of the hands.



To confirm the diagnosis incisional biopsy was performed. The specimen was taken from left buccal area in the center and edge of the lesion.

The histopathological result exhibited characteristics of LP with absence of malignant elements. To improve and stabilize the disease, the patient underwent a new pharmacological treatment plan. This plan was designed to heal the lesions, to maintain long ime results and to improve the quality of life for the patient. The therapy consisted in the use of fluocinolone acetonide 0.01% w/ lidocaine chlorhydrate 2% cream for local application directly in the affected area, mouth wash with chlorhexidine 0.1% and regenerative gel with amino acids and hyaluronic acid for local application directly in the affected area.

Corticosteroid was left to act for 30 minutes, while regenerative gel for 1 hour. In the evening, mouth wash with chlorhexidine 0.1% was done between these medications for 1 month. During the application of the drugs, the patient did not consume food or beverages. Dermatological preparations were applied in clean mucosa. The purpose of this combination consists in reducing inflammation and bacterial load and to stimulate tissue regeneration. To maximize the therapeutic efficiency and minimize side effects due to systemic absorption of corticosteroid, a de-escalated therapy was carried out (Tab.1).

Period (months)	Prescription	Usage
0-1	Fluocinolone acetonide 0.01% w/ lidocaine chlorhydrate 2%	3 times/day
	Mouth wash with chlorhexidine 0.1%	1 time/evening
	Regenerative gel with amino acids and hyaluronic	3 times/day
1-3	Fluocinolone acetonide 0.01% w/ lidocaine chlorhydrate 2%	2 times/day
	Regenerative gel with amino acids and hyaluronic	2 times/day
3-5	Fluocinolone acetonide 0.01% w/ lidocaine chlorhydrate 2%	1 time/day
	Regenerative gel with amino acids and hyaluronic	1 time/day
5-6	Fluocinolone acetonide 0.01% w/ lidocaine chlorhydrate 2%	1 time/3 days
	Regenerative gel with amino acids and hyaluronic	1 time/3 days

Tab.1. Therapy mode distributed in the duration of treatment.

RESULTS

To assess the impact of pharmacological treatment in the progress of the disease, we used the scoring system proposed by Kaliakatsou et al. (2002) (5) which includes the visual analog system for measuring the pain degree (Tab.2).

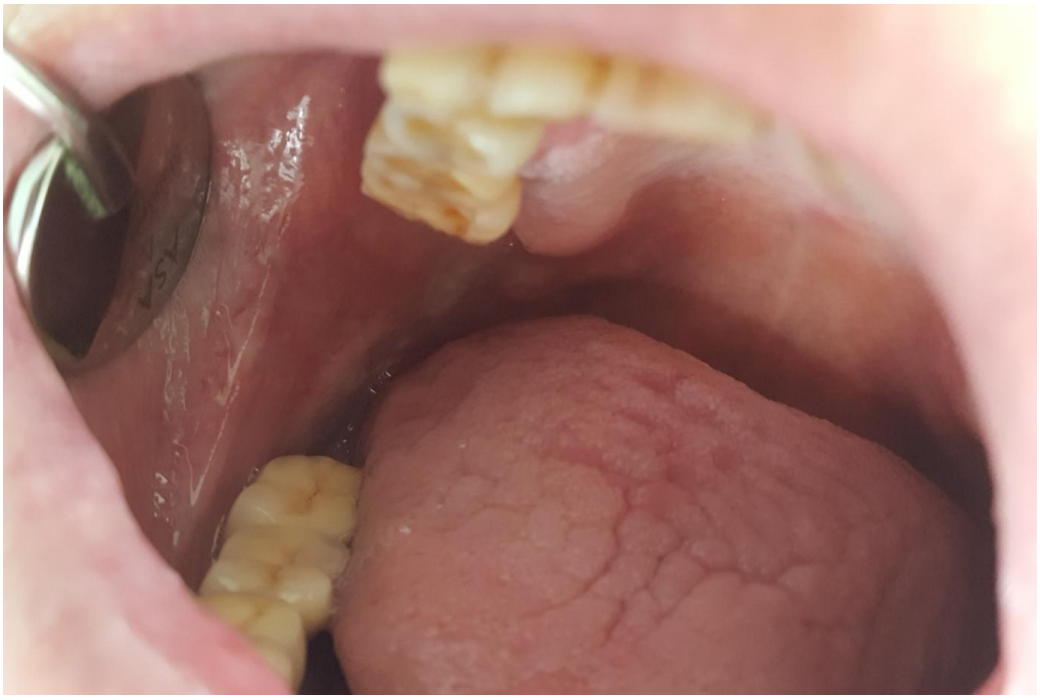
In the first clinical checkup, 10 days after the treatment plan had begun, the patient reported reduction up to 80% of pain and burning sensation and decreased lesions dimensions were noticed. In the second checkup, 1 month after the beginning of the treatment, the patient reported a complete absence of pain and burn sensation and was able to enjoy food again.

Period (months)	Evaluation of lesions	Evaluation of symptoms	
		VAS quiescence	VAS during nutrition
0	3	1	5
1	2	0	2
3	2	0	0
6	1	0	0

Tab. 2. Disease scoring system proposed by Kaliakatsou et al. (2002)*

*0= no lesion; 1= white striae only; 2= white striae and erosion ≤ 1 cm^2; 3= white striae with erosion ≥ 1 cm^2;4= white striae with ulceration ≤ 1 cm^2; 5= white striae with ulceration ≥ 1 cm^2. *Visual Analog Scale*: from 0 to 10; 0 – absence of pain, 10 – most intense pain.

Fig.3. Buccal lesion 6 months after the beginning of the treatment.



Erosive areas on buccal surfaces were healed. However, slightly erythematous areas were noticed. Gingival lesion showed slower improvements. Following further checkups, respectively 3 and 5 months after the beginning of the treatment, pain and burning sensation during food was reduced and completely lacking in quiescent state. During these clinical evaluations, the dimensions of buccal lesions decreased steadily while gingival lesion presented a slower improvement. In the last clinical evaluation, 6 months after the beginning of therapy, pain and burning sensation was absent (Fig.3).

Buccal lesions were healed and gingival lesions had not improved since the last evaluation. During this period, cutaneous lesion showed no changes. In addition, no allergic reaction or side effect of medications was noticed. However, the patient would be monitored thereafter.

DISCUSSION

Lichen planus constitutes an autoimmune pathology with an unclear etiology. Lymphocytic inflammatory infiltrate invades epithelial tissues and promote apoptosis of epithelial cells causing a chronic inflammation. Although the literature reports it as a very frequent disease (6), diagnosed cases in our institution are few. Statistical studies in our country are limited. In our case, the patient presents a particular age compared to the average age of diagnosis reported in literature. Although causative factors are not clear, Ismail et al. (7) reports a list of aggravating elements for the OLP (Oral Lichen Planus) and lichenoid reactions such as drugs (anti-malaria, diuretics, gold salts, antiretroviral) dental materials (amalgam, composites and resinous materials, metals), chronic diseases of the liver and Hepatitis C virus and genetic influence. In our case, none of the above-mentioned factors is present. Besides them, stress has been identified as one of the most frequent causes for the deterioration of the disease. A recent study showed that patients with LPO exhibit higher levels of anxiety and depression as compared to the control groups (8). In our patient, depression, anxiety and stress test (DASS) resulted negative for depression and anxiety, and very low stress levels. Furthermore, the patient reported a quiet life without major concerns. LPO cases with minimal involvement of the skin consist in 15% of all cases. Whereas, there are very few reports of cases of LP of the skin and oral cavity along (9). In our patient, the oral lesions were bilateral, multiple and with irregular borders. As well, we noticed a minimal involvement of the hands and wrists. In the scientific community, there is no consensus for the potential of malignancy of LP (9). Studies show that erosive and atrophic forms have a higher chance of malignancy which ranges from 0.3% to 12.5% according to different assessment criteria used by various authors (10). Development of squamous cell carcinoma (SCC) can occur in areas directly involved with OLP, as well as in other parts of the mucous membranes (10, 11). However, it is

not clear whether the lesion itself has intrinsic potential for malignant transformation or external factors encourage this process (12). In addition, some authors suggest that due to misunderstandings, misinterpretations and an incorrect diagnosis of this pathology, the malignant tendency of this disease was legitimized with time. Most of the cases in which oral lesions have undergone transformations, constitute patients with known history regarding exposure to these factors. Others pose mistakes in diagnosis and some fail to prove the presence of LP during transformation. Apart from these reasons, major errors lie in the incorrect identification of changes in epithelial cells during microscope evaluation (13). In our case, the patient underwent an incisive biopsy. It was the first biopsy during the entire progression of the pathology. Results presented a lesion with histological features of LP and without the presence of malignant elements. Subsequently, we can affirm that during these years there was no evidence of malignant transformation and the actual erosive lesions, showed no histological malignant characteristics. However, in some case reports, erosive forms present abnormal epithelial features under a microscope and as such they may be more susceptible to the carcinogenic transformation. For this reason, patients who present these lesions should be kept under continuous observation (13).

Management of patients with LPO is very important. Due to the chronic nature of the disease, it affects the quality of life of the patients. In general, the observed therapeutic plans in related studies, tend to be aggressive and for a short time (14). Hence, some authors are convinced that LP is a precancerous disease, thus justifying a more aggressive therapy in order to give a counter response to the disease and autoimmune inflammation (14,15). Since this disease is not curable, we can only focus on improving symptoms and the quality of life of the patient. Therefore, we focused on a de-escalating therapy, with low potency corticosteroid but extended in time. Being aware of the fact the fact that immunity is a dynamic system, we believe that a 'coexistence' and 'friendly' therapy against autoimmune lesions would be a mutual language to achieve and maintain the desired result for a longer time in the post therapy period. The fact that previous topical pharmacological therapies with corticosteroids of class I (powerful) and class IV (medium power) have resulted in an even more aggressive recurrence of lesions, shows that a possibility of the management of this disease might be a coexistence with the disease (16). Pharmacological drug choices are various and include ointments with clobetasol propionate, dexamethasone, betamethasone valerate, triamcinolone acetonide, fluocinolone acetonide, fluocinonide and hydrocortisone. A low potency corticosteroid of class 6 as fluocinolone 0,01% (17) was chosen. The effects showed to be slower, compared with high potency corticosteroids. However, side effects caused by

systemic absorption were absent. Our concept is a gradual reduction of inflammation and maintaining long-lasting positive effects, with minimal adverse effects. Consequently, a balance of the immune system, which over time will lean toward a gradual recovery and sustainable results

CONCLUSIONS

In oral lichen planus, identification and elimination of stimulant and influencing factors is very important. Improvement of symptoms is almost always achieved with the use of local corticosteroids. Since the treatments are not specific, they aim at eliminating the inflammation and therefore they are partially successful. Although possibilities for a malignant transformation are small, patients should be kept under a long-term supervision, particularly those who represent erosive lesions. Besides pharmacological treatment, maintaining oral hygiene and particularly the cooperation of the patient are essential factors in the successful management of the disease.

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