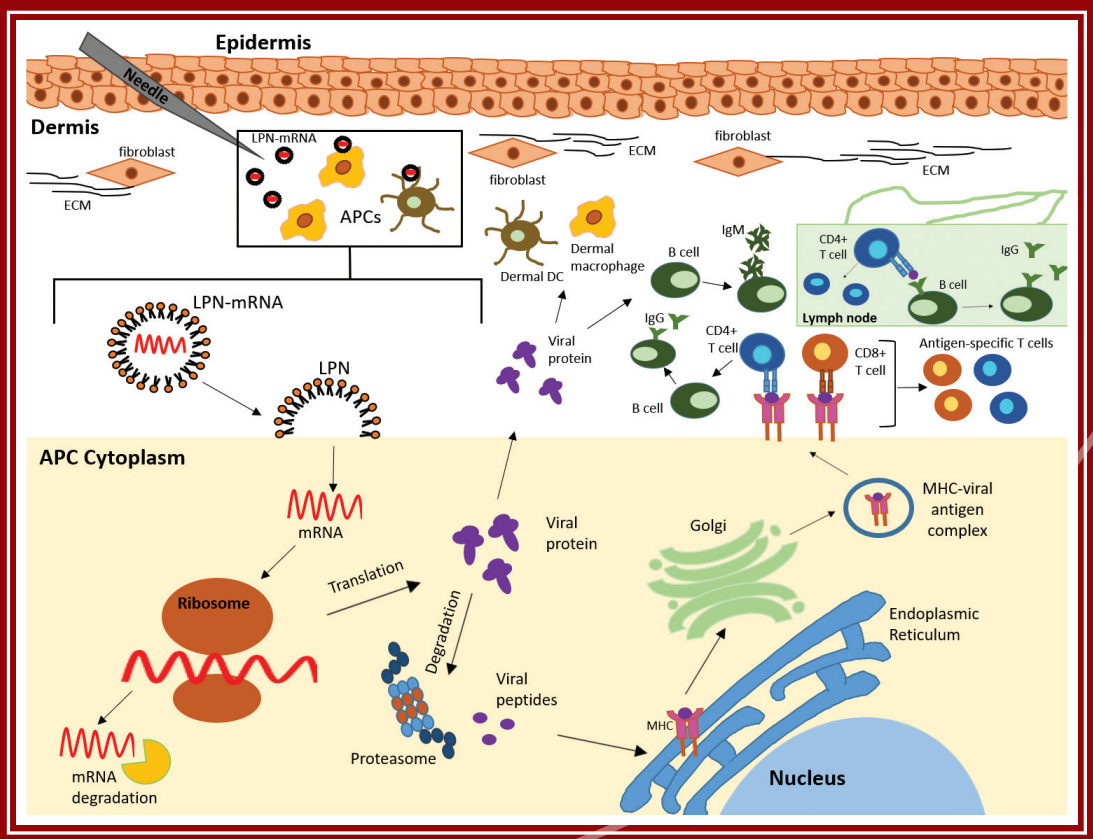
VOLUME 40 • ISSUE 1 • JANUARY - MARCH 2021

Achaiki Iatriki

OFFICIAL PUBLICATION OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS



Proposed mechanism of COVID-19 mRNA vaccines.

ISSN: 1106-3319
ISSN (ON LINE): 1792-3018

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

GENERAL INFORMATION

ISSN Print Edition: 1106-3319 **Journal Homepage:** http://www.iedep.gr/

ISSN Electronic Edition: 1792-3018 **NLM Unique ID:** 9802550

Journal citation: *Achaiki Iatriki* is published on behalf of the Journal of the Medical Society of Western Greece and Peloponnesus (IEDEP), representing the Society's official Journal. Please cite articles of the Journal as: Author names. Title of article. Ach Iatriki year;volume:pages.

Aims and scope: The journal publishes original papers on clinical and basic research from all areas of the health sciences including healthcare. *Achaiki Iatriki* is an open access journal. It provides immediate free access to its scientific contents and authors are not charged for submission, processing or publication of the manuscripts.

Abstracting and indexing services: Achaiki Iatriki is abstracted/indexed in the following databases: Mulford Health Science Library, Index Copernicus, Google Scholar, the Greek IATROTEK and National Library of Medicine.

MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS

42 Votsi Street, Patras 26221, Greece

Tel: +30 2610 279579, Fax: +30 2610 220518

email: iede_pel@yahoo.gr

Publisher

Medical Society of the Western Greece and Peloponnesus

Editor-in-Chief

Christos Triantos

email: achaiki.iatriki@gmail.com

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

ACHAIKI IATRIKI

OFFICIAL JOURNAL OF THE MEDICAL SOCIETY OF WESTERN GREECE AND PELOPONNESUS (IEDEP)

ACHAIKI IATRIKI

Quarterly Official Journal of the
Medical Society of Western Greece And Peloponnesus (IEDEP)

CONTENTS

Dear colleagues,

In the current issue, you will find emerging new data on the novel coronavirus SARS-CoV-2 and COVID-19. The editorial by Tourkochristou et al. presents a comprehensive update on mRNA vaccines. The editorial by Ntouvas et al. describes the association of COVID-19 with the development of venous thromboembolic events as well as hemorrhagic complications. Lastly, the original article by Rodi et al. reports a protocol for RNA extraction for SARS-CoV-2 detection.

In addition, this issue includes an editorial by Fragkou et al. which discusses the management of abnormal liver tests in primary care, a common laboratory finding related to liver diseases. Konstantakis et al. describe the management of acute pancreatitis according to various algorithms. Akinosoglou et al. focus on the use of information reported by patients (patient reported outcomes - PRO), aiming to evaluate treatment outcomes by health professionals, in order to understand and improve the quality of healthcare services. Markantes et al. present the new advances in the management of differentiated thyroid cancer and conclude that there is a great need for the development of novel, targeted therapies based on molecular data. Lastly, the editorial by Androutsopoulos et al. presents all relevant aspects regarding staging and treatment options of endometrial cancer, pointing out that systematic surgical staging remains the initial therapeutic approach in endometrial cancer patients.

The original article by Maliouki et al. evaluates the use of Non-Invasive Ventilation (NIV) with continuous positive airway pressure (CPAP) mode in critically ill patients with acute or chronic respiratory problems in a tertiary general hospital in Greece. Another original study by Bartsokas et al., investigates the distribution differences regarding the geographic location and population size between primary sector and hospitals, in the middle of economic crisis, in the Western Greece. Solomou et al. review imaging findings of upper renal tract infection, especially abscess formation, with an emphasis on US and MRI imaging, in the work-up of complicated pyelonephritis in young women.

The first review article, by Mastorakou et al. presents a comprehensive overview on health risks from radiation, focusing on electromagnetic radiation and suggests precautions instructions and the use of protection measures to public. Lastly, the second review by Zarkada et al. summarizes the most recent evidence-based data related to paediatric feeding and swallowing disorders and in parallel, focuses on the Speech and Language Pathologist's perspective on paediatric dysphagia.

C. Triantos
Assistant Professor in Internal Medicine
and Gastroenterology Faculty of Medicine,
School of Health Sciences, University of Patras
Editor-in-Chief of the journal "ACHAIKI IATRIKI"

mRNA vaccines to protect against COVID-19

Evanthia Tourkochristou[1,2], Athanasia Mouzaki[1,3]

PREFACE

The outbreak of coronavirus disease (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), in Wuhan, China, spread rapidly around the world and was declared a pandemic by the World Health Organization on March 11, 2020. To date, 97.5 million cases and 2,089,460 deaths have been reported worldwide (Johns Hopkins Coronavirus Resource Center. COVID-19 Map. Accessed January 22, 2021. https://coronavirus.jhu.edu/map.html). The high transmissibility of SARS-Cov-2, which causes different clinical symptoms that escalate to severe, fatal infection in certain cases, made the rapid development of vaccines urgent [1]. The development of mRNA vaccines against SARS-Cov-2 was a strategy that gained rapid acceptance because their production is simple, rapid, and inexpensive - features that can save time against the rapidly spreading COVID -19 pandemic [2].

Previous attempts for mRNA vaccine creation and use

mRNA vaccines have already been designed and tested in preclinical studies for a variety of infectious diseases such as Zika, influenza, papilloma, CMV, Ebola, rabies and HIV viruses, with varying results [4]. mRNA vaccines against chikungunya, hMPV/PIV3, H10N8, and H7N9 viruses were tested in phase I clinical trials and were found to be safe and well tolerated, eliciting robust antibody responses and causing mild side effects at the injection site and systemically [3-5]. A rabies virus glycoprotein (RABV-G) mRNA vaccine caused mild to moderate injection site side effects in almost all vaccinated subjects, while systemic side effects such as fever, fatigue, and pain occurred in 78% of subjects. Nevertheless, the vaccine failed to induce a sufficient immune response as antibody titers dropped 1 year after vaccination [6]. Lower antibody titers were also observed in clinical trials than in animal studies when influenza virus-specific mRNA vaccines were used [5]. An mRNA vaccine against HIV was studied in a phase I trial in chronically HIV-infected patients; the vaccine induced moderate HIV-specific T-cell responses and mild side effects [7]. Studies on optimal dosage, delivery systems and routes of administration are still ongoing to improve the immunogenic efficacy of mRNA vaccines and ensure their good safety profile.

Formulation and action of mRNA vaccines

A conventional mRNA-based vaccine is prepared in a simple, rapid, and effective manner in vitro by transcribing linear plasmid DNA using a T7, a T3, or a Sp6 phage RNA polymerase. The transcript, which consists of an open reading frame encoding the target protein, flanking untranslated regions UTRs and a poly(A) tail, is capped at the 5′ end and purified.

Another type of mRNA platform, called self-amplifying mRNA (samRNA), is based on an engineered genome of alphaviruses that contains genes encoding the target antigen and RNA replication machinery [8]. samRNAs are able to direct their intracellular self-replication, resulting in the synthesis of multiple copies of the target protein in a manner that mimics the de novo production of viral antigens [8]. Lipid nanoparticles (LPNs) are effective transporters for engineered mRNA sequences, ensuring their stability and integrity; however, it is still unclear whether their components

[1]Division of Hematology, Department of Internal Medicine
[2]Division of Gastroenterology, Department of Internal Medicine,
[3]Laboratory of Molecular Diagnosis of Infectious Agents
Medical School, University of Patras, Patras, Greece

Received: 26 Dec 2020; Accepted: 23 Jan 2021

Key words: *SARS-CoV-2; BNT162b2; mRNA-1273; mechanism; clinical trials*

can be toxic [9]. A proposed mechanism of mRNA vaccines is shown in Figure 1. Once the LPN-encapsulated mRNA enters the cytosol of an antigen-presenting cell (APC) via endocytosis, it uses the host cell's translational machinery to drive translation of the target protein, which then undergoes post-translational modifications to become a properly folded, functional protein. The translated target antigen is either degraded by the proteasome within the cell, where the antigen peptides are transported into the endoplasmic reticulum and loaded onto MHC molecules presenting them on the cell surface to induce antigen-specific T-cell responses, or secreted by the host cell and re-captured by other APCs (macrophages, dendritic cells, B-cells). The vaccine mRNA is eventually degraded by the natural intracellular processes [10].

mRNA vaccines against COVID-19

Two samRNA vaccines against SARS-Cov-2, the BNT162b2 (Biotech/Pfizer) and the mRNA-1273 (Moderna), have reached phase III clinical trials. The mRNA-1273 is an LNP-encapsulated mRNA vaccine that encodes the S-2P antigen through which SARS-Cov-2 enters the host cell. This is the first SARS-Cov-2 vaccine to enter phase I clinical trials on March 16, 2020. mRNA-1273, administered in 2 doses, showed mild to moderate adverse effects after both vaccinations, eliciting time- and dose-dependent antibody responses against the S-2P

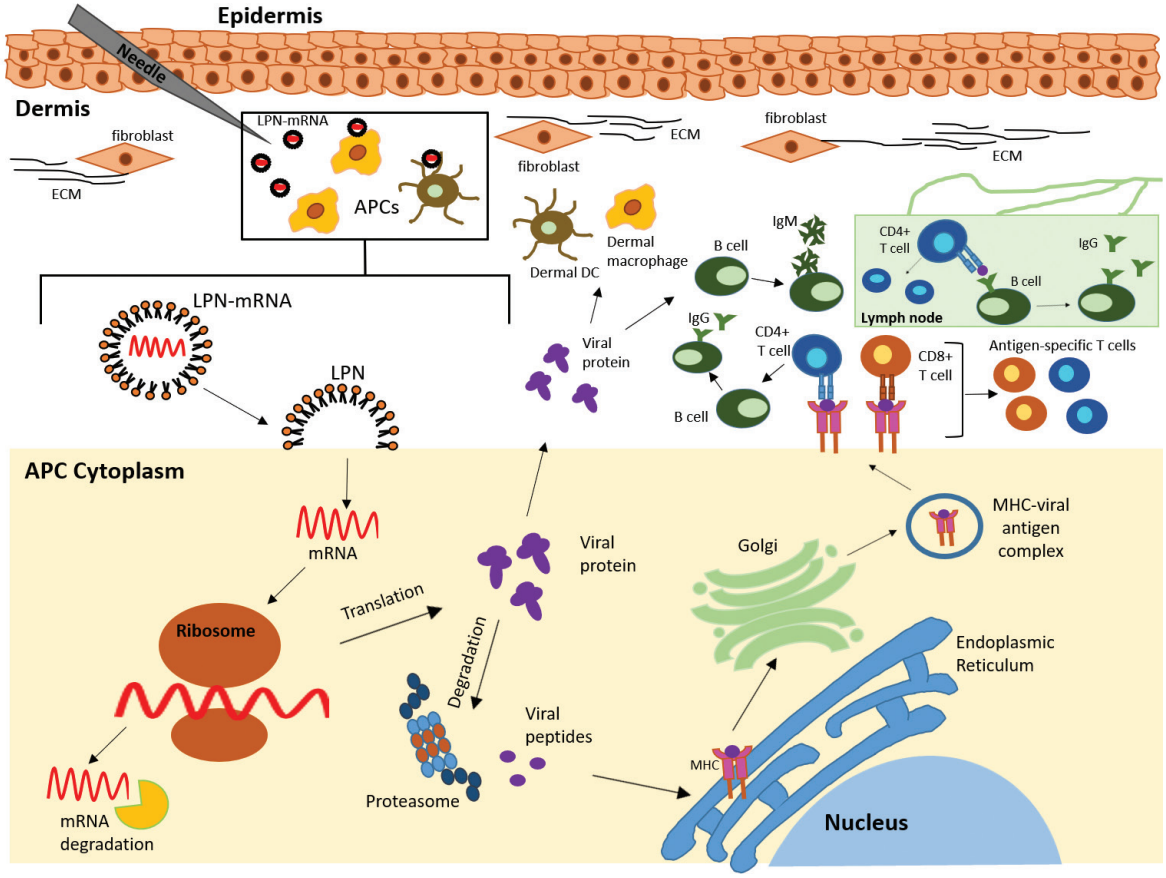


Figure 1. Proposed mechanism of antiviral mRNA vaccine. Intramuscular injection of the vaccine releases mRNA (LPN-mRNA) encapsulated in lipid nanoparticles in the dermis. The LPN-mRNA is taken up by antigen-presenting cells (APCs), including dermal dendritic cells (DCs) and dermal macrophages. Once the LPN-mRNA enters the cytoplasm of the APC by endocytosis, it uses the host cell's translational machinery to direct the translation of viral proteins (antigens). After synthesis, the viral proteins are properly folded and made functional by post-translational modifications. The translated viral antigens are then degraded by the proteasome within the cell, resulting in antigenic peptides. The peptides are transported into the endoplasmic reticulum and loaded onto MHC (HLA) molecules and presented on the cell surface to naive CD4+ T helper and CD8+ T cytotoxic cells, which become activated antigen-specific T-cells. Antigenic peptides secreted by the host cell are recaptured and re-presented by other APCs. In parallel, they are recognized by naive B-cells in the periphery, which are activated and differentiate into IgM-producing plasmocytes. B-cells that capture antigenic peptides and present them to antigen-specific T helper cells are licensed by these to differentiate and produce isotype-switched antigen-specific antibodies (mainly IgG) in the lymph nodes.

protein, antigen-specific CD4+ T-cell responses, and to a lesser extent CD8+ T-cell responses [11]. Neutralizing antibodies were observed in all participants only after the second vaccination, highlighting the need for administration of two doses. Induction of antibody and Th1 T-cell responses represents a major goal for SARS-CoV-2 vaccines to reduce the risk of vaccine-associated enhanced respiratory disease observed in previous animal studies of SARS-CoV and MERS-CoV infections [12]. A phase III study of 30,420 participants (aged 18 to 65 years) who received the vaccine in two intramuscular doses showed that the efficacy of the vaccine against COVID-19 starting 14 days after the second dose was estimated to be 94.1%, although lower in older participants (>65). Mild local reactions and transient moderate-to-severe systemic events, including fatigue, myalgia, arthralgia, and headache, as well as a low frequency (<2%) of non-fatal serious adverse events and hypersensitivity reactions were observed after the second dose of mRNA-1273 [13].

However, there are still limitations, such as the short follow-up time of the safety and efficacy of the vaccine, as the study is not yet completed and a follow-up of 2 years will be conducted. The FDA granted an Emergency Use Authorization (EUA) for the use of Moderna vaccine mRNA-1273 in adults over 18 years of age in the United States on December 18, 2020, and the European Commission approved the use of Moderna vaccine mRNA-1273 in adults over 18 years of age in Europe on January 6, 2021.

BNT162b2 (BioNTech/Pfizer) is another LPN-formulated mRNA vaccine encoding a prefusion-stabilized membrane-anchored full-length SARS-CoV-2 spike protein. Two phase I/II clinical trials were conducted in the United States and Germany to evaluate the safety and immunogenicity profile of a 2-dose regimen (1, 10, 20, or 30 μg per dose) of BNT162b2 in healthy participants aged 19 to 85 years [14,15]. Two doses of 30μg BNT162b2 achieved a high production of SARS-Cov-2 neutralizing antibody titers (lasting up to 63 days after the boost vaccination) and elicited antigen-specific CD8+ T-cell and Th1-type CD4+ T-cell responses. However, lower antibody responses were observed in older compared to younger participants, a result also observed in other vaccines, and is attributed to immune senescence [16,17]. BNT162b2 entered phase II/III studies (NCT04368728) including 43,448 participants 16 to 55 years or older, assigned 1:1 to receive an intramuscular injection of 30 μg BNT162b2 or placebo in a 2-dose schedule, 21 days apart. A total of 37,706 participants from various ethnic groups, including individuals with obesity and coexisting conditions, and with no evidence of existing or prior SARS-CoV-2 infection were studied for at least 2 months post-vaccination. The efficacy of BNT162b2 was 95% because only 8 cases got COVID-19 among the 36,523 participants after the 2nd dose of vaccination compared to 162 cases that received the placebo [18]. During the clinical evaluation of BNT162b2, a good safety profile was observed, characterized by mild to moderate local reactions in all participants (predominantly injection site pain and injection site redness or swelling, which occurred less frequently) that resolved within 1 to 2 days and did not recur after the second dose. Systemic events (fatigue, headache, chills, fever, muscle/joint pain) after both vaccinations, occurred on day 2 after vaccination and resolved within 5 days. Fever (>38oC), fatigue, and headache were more common in younger participants (16 to 55 years old) than in older ones (>55 years old) and mainly after the second dose of vaccination. A transient slight increase in CRP level and a decrease in blood lymphocytes were also observed. CRP levels and lymphocyte counts are considered pharmacodynamic markers of the mode of action of RNA vaccines, and the transient decrease in lymphocytes is probably due to the observed redistribution of lymphocytes to lymphoid tissues related to the stimulation of the innate immune system, according to previous clinical data on RNA vaccines [19]. BNT162b2-related serious adverse events were reported in a few participants, including postvaccination shoulder injury, right axillary lymphadenopathy, paroxysmal ventricular arrhythmia, and right leg paresthesia; serious systemic events occurred in less than 4% of participants. Although 2 BNT162b2 and 4 placebo deaths occurred, none were related to vaccine or placebo administration. Safety monitoring will continue for 2 years after the second dose. The encouraging results of the Phase II/III trials allowed the FDA to grant BioNTech/Pfizer an emergency approval for BNT162b2 on December 11, 2020 and the European Commission to grant BioNTech/Pfizer a conditional marketing authorization (CMA) on December 21, 2020.

CONCLUSIONS

Future considerations include evaluation of adverse events longer than 2 months after boost vaccination, duration of vaccine protection, vaccine protection in younger adolescents, children, pregnant women, immunocompromised individuals, individuals with

asymptomatic infection, and individuals with a history of coronavirus. In addition, ongoing studies need to optimize RNA formulation and stability to reduce the need for cold storage (the vaccine is currently stored and shipped at -60 to -80oC) and improve vaccine efficacy, as the nucleotide composition of RNA may affect its immunostimulatory activity and reactogenicity profile [20].

Overall, given the relatively good safety and immunogenicity profiles of mRNA vaccines, there is optimism that global vaccination will help end the pandemic COVID-19. Hopefully, if this type of vaccine proves effective, there will be renewed efforts to use this technology to produce vaccines for the many infectious diseases that remain incurable.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *ET is a recipient of Karatheodoris grant #80672 from the University of Patras.*

REFERENCES

1. Sun K, Chen J, Viboud C. Early epidemiological analysis of the coronavirus disease 2019 outbreak based on crowdsourced data: a population-level observational study. Lancet Digit Health. 2020; 2(4):e201-e208.
2. Jackson NAC, Kester KE, Casimiro D, Gurunathan S, DeRosa F. The promise of mRNA vaccines: a biotech and industrial perspective. NPJ Vaccines. 2020; 5:11.
3. Moderna Announces Positive Interim Phase 1 Data for First Combination Vaccine Against the Respiratory Viruses hMPV and PIV3. 2019. Available from: https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-positive-interim-phase-1-data-first
4. Shaw C, Panther L, August A, Zaks T, Smolenov I, Bart S, et al. Safety and immunogenicity of a mRNA-based chikungunya vaccine in a phase 1 dose-ranging trial. Int J Inf Dis. 2019; 79(S1):17.
5. Bahl K, Senn JJ, Yuzhakov O, Bulychev A, Brito LA, Hassett KJ, et al. Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses. Mol Ther. 2017; 25(6):1316-1327.
6. Alberer M, Gnad-Vogt U, Hong HS, Mehr KT, Backert L, Finak G, et al. Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. Lancet. 2017; 390(10101):1511-1520.
7. Leal L, Guardo AC, Morón-López S, Salgado M, Mothe B, Heirman C, et al. Phase I clinical trial of an intranodally administered mRNA-based therapeutic vaccine against HIV-1 infection. AIDS. 2018; 32(17):2533-2545.
8. Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018; 17(4):261-279.
9. Reichmuth AM, Oberli MA, Jaklenec A, Langer R, Blankschtein D. mRNA vaccine delivery using lipid nanoparticles. Ther Deliv. 2016; 7(5):319-334.
10. Wadhwa A, Aljabbari A, Lokras A, Foged C, Thakur A. Opportunities and Challenges in the Delivery of mRNA-based Vaccines. Pharmaceutics. 2020; 12(2):102.
11. Jackson LA, Anderson EJ, Rouphael NG, Roberts PC, Makhene M, Coler RN, et al. An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. N Engl J Med. 2020; 383(20):1920-1931.
12. Corbett KS, Edwards DK, Leist SR, Abiona OM, Boyoglu-Barnum S, Gillespie RA, et al. SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature. 2020; 586(7830):567-571.
13. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, et al. Efficacy and Safety of the mRNA-1273 SARS-CoV-2 Vaccine. N Engl J Med. 2020; (in press). doi: 10.1056/NEJMoa2035389
14. Walsh EE, Frenck RW, Jr., Falsey AR, Kitchin N, Absalon J, Gurtman A, et al. Safety and Immunogenicity of Two RNA-Based COVID-19 Vaccine Candidates. N Engl J Med. 2020; 383(25):2439-2450.
15. Sahin U, Muik A, Vogler I, Derhovanessian E, Kranz LM, Vormehr M, et al. BNT162b2 induces SARS-CoV-2-neutralising antibodies and T cells in humans. medRxiv. 2020. doi: https://doi.org/10.1101/2020.12.09.20245175
16. Muñoz N, Manalastas R, Jr., Pitisuttithum P, Tresukosol D, Monsonego J, Ault K, et al. Safety, immunogenicity, and efficacy of quadrivalent human papillomavirus (types 6, 11, 16, 18) recombinant vaccine in women aged 24-45 years: a randomised, double-blind trial. Lancet. 2009; 373(9679):1949-1957.
17. Boraschi D, Del Giudice G, Dutel C, Ivanoff B, Rappuoli R, Grubeck-Loebenstein B. Ageing and immunity: addressing immune senescence to ensure healthy ageing. Vaccine. 2010; 28(21):3627-3631.
18. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA COVID-19 Vaccine. N Engl J Med. 2020; 383(27):2603-2615.
19. Kamphuis E, Junt T, Waibler Z, Forster R, Kalinke U. Type I interferons directly regulate lymphocyte recirculation and cause transient blood lymphopenia. Blood. 2006; 108(10):3253-3261.
20. Kondili M, Roux M, Vabret N, Bailly-Bechet M. Innate immune system activation by viral RNA: How to predict it? Virology. 2016; 488:169-178.

Corresponding author:

Athanasia Mouzaki
Laboratory of Molecular Diagnosis of Infectious Agents, Medical School, University of Patras, Patras, GR-26500, Greece
Tel.: +30 2610 969123,
E-mail: mouzaki@upatras.gr
ORCID: 0000-0001-5548-7002

Covid-19 and Thromboembolic events

Ioannis Ntouvas[1], Chrysanthi Papageorgopoulou[2], Konstantinos Nikolakopoulos[2]

The coronavirus pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been reportedly associated with a high risk of thrombotic complications. It is well established that viral infections are linked to coagualation alterations as part of an interaction between the immune system and blood coagulation. Both systems are closely intertwined for an effective immune response that limits the infection. The key point is the recruitment of leukocytes simultaneously with the activation of coagulation where clot components, such as fibrin, serve as a scaffold for cell adherence and migrations [1]. On the other hand, pathogens such as viruses may induce tissue factor (TF) expression, a major activator of coagulation, on monocytes and endothelial cell surfaces. Therefore, there is an activation of coagulation that could lead to both Venous Thromboembolic Events (VTE) as well as hemorrhagic complications [2]. It is not well established why some viruses cause hemorrhages (e.g Ebola), others thrombose (e.g. cytomegalovirus, SARS-CoV-2) and others both complications (e.g. varicella zoster virus).

Many studies suggest that there is a substantial increase of thromboembolic events in Covid-19 patients. Hence, in a large study from Milan, including 388 patients, 61 of whom were admitted to the Intensive Care Unit (ICU), the reported VTE rate was 7.7%. VTE incidence was higher in ICU patients (16.7% versus 6.4%). It is worth mentioning that half of the thromboembolic events were diagnosed within 24 h of hospital admission, raising the question whether thrombosis is an early complication of Covid-19 or a determinant of further deterioration [3]. In a retrospective study at the Amsterdam University Medical Center, including 198 patients (74 in ICU and 124 on medical ward), 33 (17%) were identified with VTE. The proportion was dramatically higher in ICU patients (39% vs 3.2%), although they had received thromboprophylaxis at standard or double doses [4]. Likewise, Klok et al. [5], found a 31% incidence of VTE in ICU patients with Covid-19, despite thromboprophylaxis. Similarly, Cui et al. [6] in 81 ICU patients with Covid-19 pneumonia, found an 25% incidence of VTE.

The increased VTE rate in Covid-19 patients, combined with an extensive alteration in biological markers (e.g. D-Dimers), suggest an abnormal hypercoagulability. In a retrospective cohort study from Wuhan-China, among hospitalized SARS-CoV-2 patients, 68% had D-dimer levels above the upper limit. Although, D-Dimer levels do not constitute a specific marker for VTE, increased levels reveal the impact of SARS-CoV-2 to coagulation. More importantly, the D-dimer increase was dynamic. It continues to rise as the disease progresses, reflecting a prognostic indicator of mortality [7]. Furthermore, in an another study, it is reported that prothrombin, a natural anticoagulant, was lower in Covid-19 patients [3]..Additionally, in a case series in France, the lupus anticoagulant was identified in 25 of 56 patients (45%) admitted with Covid-19 [8].

Covid-19, compared with other bacterial or viral infections, seems to have a stronger correlation with VTE. The first 107 consecutive Covid-19 patients admitted to ICU in a single center in France, were compared to patients admitted to the same ICU one-year prior with influenza and other diseases. At the time of analysis, 22 (20.6%) of the Covid-19 patients had Pulmonary Embolism (PE). In contrast, one year prior, the general and influenza ICU population, had PE rates of 6.1% and 7.5%, respectively [9]. In another, multicenter study from the Netherlands, the incidence of thrombotic complications in hospitalized Covid-19 patients were

[1]General Hospital of Patras, Patras, Greece
[2]Department of Vascular Surgery, University Hospital of Patras, Patras, Greece

Received: 14 Sep 2020; Accepted: 23 Dec 2020

Key words: *Covid-19; thromboembolic events; ICU*

considerably higher compared to that of hospitalized influenza patients, suggesting a possible SARS-COV-2 specific effect [10]. Furthermore, a prospective cohort study from France, which included 150 Covid-19 patients, with Acute Respiratory Distress Syndrome (ARDS) and 233 ARDS patients due to bacterial and other viral infections, in 4 ICUs, revealed that Covid-19 patients had statistically significant higher rates of PE (11.7% versus 2.1%) [11].

Another point of view, regarding the VTE and Covid-19 correlation is that according to several studies, there is a disproportionate high number of PE not related to deep vein thrombosis [12]. This emerges a hypothesis that the pathophysiology of the pulmonary thrombotic events in Covid-19 may not be embolic at all, which in turn could have major implications for treatment. A review of 10 autopsies of Covid-19 patients (5 men, 5 women) found evidence of microthrombi in lung tissue, thus raising the suspicion that in-situ pulmonary thrombosis may be the main pathophysiological mechanism [13].

In conclusion, Covid-19 is a very contagious virus, with a high proportion of thromboembolic events. Patients admitted to ICU are in a higher risk for VTE, despite thromboprophylaxis. Therefore, it is of vital importance to be alert for VTE, when treating Covid-19 patient admitted to hospital, especially in the ICU.

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare*

REFERENCES

1. Antoniak S. The Coagulation system in host defence. Res Pract Thromb Haemostat. 2018;242(3):549-557.
2. Goeijenbier M, van Wissen M, van de Weg C, Jong E, Gerdes VEA. Meijers JCM, et al. Review: Viral Infections and Mechanisms of Thrombosis and Bleeding. J Med Virol. 2012;84(10):1680-96.
3. Lodigiani C, Iapichino G, Carenzo L, Cecconi M, Ferrazzi P, Sebastian T, et al. Venous and arterial thromboembolic complications in COVID-19 patients admitted to an academic hospital in Milan, Italy. Thromb Res. 2020;191:9-14.
4. Middeldorp S, Coppens M, van Haaps TF, Foppe M, Vlaar AP, Müller MCA, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020;18(8):1995-2002.
5. Cui S, Chen S, Li X, Liu S, Wang F. Prevalence of venous thromboembolism in patients with severenovel coronavirus pneumonia. J Thromb Haemost, 2020; 18(6):1421-1424.
6. Klok FA, Kruip MJHA, van der Meer NJM, ArbousMS, Gommers DAMPJ, Kant KM, et al. Incidence of thrombotic complications in critically ill ICU patients with COVID-19. Thromb Res. 2020;191:145-147
7. Han H, Yang L, Liu R, Liu F, Wu K, Li J, et al. Prominent changes in blood coagulation of patients with SARS-CoV-2 infection. Clin Chem Lab Med. 2020;58(7):1116-1120.
8. Harzallah I, Debliquis A, Drenou B. Lupus anticoagulant is frequent in patients with Covid-19. J Thromb Haemost. 2020;18(8):2064-2065.
9. Poissy J. Goutay J, Caplan M, Parmentier E, Duburcq T, Lassalle F, et al. Pulmonary embolism in COVID-19 patients:awareness of an increased prevalence. Circulation. 2020;142(2):184-186.
10. Stals MAM, Grootenboers MJJH, van Guldener, C Kaptein FHJ, Braken SJE, Chen Q, et al. Higher Incidence of Thrombotic Complications in Hospitalized Patients with SARS-COV-2 Virus versus Influenza Virus Infections. ISTH Virtual Congress; 2020 July 12-14.
11. Helms J, Tacquard C, Severac F, Leonard-Lorant I, Ohana M, Delabranche X. High risk of thrombosis in patients in severe SARS-CoV-2 infection: A multicenter prospective cohort study. Intensive Care Med. 2020;46(6):1089-1098.
12. Cattaneo M, Bertinato EM, Birocchi S, Brizio C, Malavolta D, Manzoni M, et al. Pulmonary embolism or pulmonarythrombosis in COVID-19? Is the recommendation to use high-dose heparin for thromboprophylaxis justified? Thromb Haemost. 2020;120(8):1230-1232.
13. Dolhnikoff M, Duarte-Neto AN, de Almeida Monteiro RA, da Silva LFF, de Oliveira EP, Saldiva PHN, et al. Pathological evidence of pulmonary thrombotic phenomena in severe COVID-19, J Thromb Haemost. 2020;18(6):1517-1519.

Corresponding author:

Ioannis Ntouvas
D. Gounari 187, Patra, 26331
Tel.: +30 6936 188344,
E-mail: ntouvas@gmail.com

ACHAIKI IATRIKI | 2021; 40(1):13–15

Management of elevated liver enzymes in primary care

Nikolaos M. Fragkou, Stefania Kiapidou, Emmanouil K. Sinakos

A 60-year old female visits her family doctor's practice for her annual check-up. Blood tests results are normal except for Aspartate and Alanine Aminotransferases (AST and ALT) (2x Upper Limit of Normal). She is 167cm tall and weighs 83kg. She has no history of Hepatitis B in her family, but she has not received a vaccination against the Hepatitis B Virus (HBV). She also reports no history of intravenous drug use and no family history of autoimmune diseases. She had received a blood transfusion in 1978 during a fracture repair surgery. She claims to consume no more than 3 glasses of wine per week.

This case vignette highlights a common clinical problem that doctors in primary care face. Current practice when managing abnormal liver tests in primary care is variable with physician strategies ranging from ignoring the results, repeating sampling, requesting additional tests or referring to specialist services. Ideally, a physician should recognize whether this elevation is clinically important and which should be the appropriate steps to clarify the etiology of this finding.

The blood tests that are used to assess liver condition include AST and ALT, Alkaline Phosphatase (ALP), Gamma-Glutamyl Transferase (γGT), bilirubin (conjugated and unconjugated), Prothrombin Time (PT) and/or International Normalized Ratio (INR), serum albumin and platelet count. These tests are generally inexpensive and are commonly used in primary care for various reasons including the exclusion of chronic liver disease, monitoring for potential adverse effects of drugs and investigation of the generally unwell patient. It must be stressed that liver chemistries including ALT, AST, ALP and γGT are markers of liver injury, not liver function, and should be referred to simply as liver tests. Liver synthetic function is reflected by the levels of albumin, bilirubin and prothrombin time which can be also influenced by extrahepatic factors. Elevation of AST and ALT signify hepatocellular injury, whereas elevation of ALP and γGT usually reflect obstruction of the biliary tree.

It is not uncommon that some of these tests produce an abnormal result. Approximately 20% of the liver tests that are performed for the first time in a patient lay outside the reference range [1]. Despite the frequency that these irregularities may occur, they are not to be overlooked. A study in Germany in 1998 by Arndt et al showed that men with elevated AST (>18 IU/L) had a three times higher risk for all-cause mortality compared with men with lower values of AST [2]. Some of the most common mistakes that occur when a patient is managed in primary care include unnecessary repeat of the tests after a short period of time, disregard of the results and unnecessary referral to specialist services. Another common error is the assumption that the magnitude of derangement of a liver test necessarily correlates with prognosis. Due to the fact that, used in isolation, the aforementioned liver blood tests neither have high specificity nor can be used as exclusion tools, it is considered appropriate that these tests are followed by more accurate diagnostic tests. To offer guidance to Primary Care Units physicians on managing abnormal liver tests, both the British Society of Gastroenterology and the American College of Gastroenterology published an upgraded set of guidelines in 2018 and 2017 respectively [3, 4]. Moreover, an artificial intelligence-based algorithm was recently developed in Scotland, possibly stating a new era for the investigation of liver diseases in primary care [5]. Based on irregular values

4th Department of Internal Medicine, Aristotle University of Thessaloniki, General Hospital of Thessaloniki "Ippokratio"

Received: 21 Feb 2020; Accepted: 05 Mar 2020

Key words: *Primary care; liver tests; transaminases; referral; hepatologist*

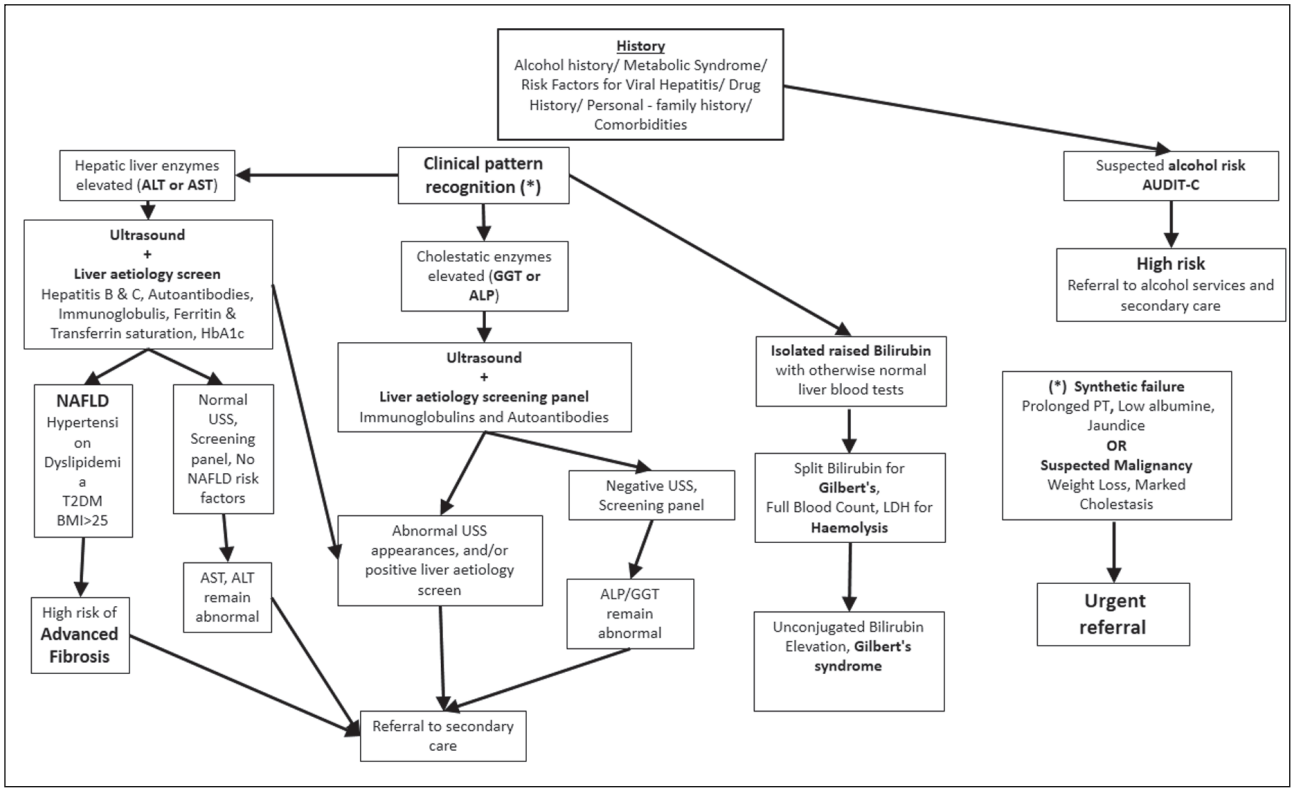


Figure 1. Diagnostic algorithm of liver tests elevation.

in liver blood tests, the algorithm automatically triggers a series of diagnostic blood tests, in order to find the cause of this irregularity. In the end, it proposes a diagnosis, which the physician has the right to accept or dismiss facilitating the whole investigation process.

During the discussion with a patient with abnormal liver blood tests, it is essential that a physician assesses the possibility that these irregularities are related to alcohol consumption. This can be aided with the use of specific questionnaires like AUDIT-C, which consists of 3 simple questions; a) how often does one drink, b) how many units of alcohol does one consume and c) how often does one "binge drink" [6]. Provided that the patient is in high risk for alcohol related liver disease and the liver synthetic function is compromised, the physician should refer him to alcohol services and to a specialist center. Apart from ethanol consumption, it is important to know whether the patient has been exposed to risk factors of contracting a viral hepatitis, such as intravenous drug use or blood transfusion before 1992. Moreover, the patient's history should include a personal and family history of liver and autoimmune diseases. Naturally, it is of paramount importance to reveal any relevant symptoms, comorbidities and concomitant medication including those purchased without medical prescription.

As shown in Figure 1, there are three distinct patterns of liver enzyme elevations. The primary care physician should be in place to correctly categorize the patient and subsequently follow the corresponding follow-up process. At any point, should the physician suspect either liver synthetic failure because of otherwise unexplained jaundice, low albumin and prolonged INR or malignancy because of weight loss or other related symptoms he should refer the patient to a specialist center urgently.

In the event that AST and/or ALT levels are elevated, as in the patient of the case vignette, a liver etiology screening panel and an upper abdomen ultrasonography should be performed. The "screening panel" should include ferritin and transferrin saturation levels, serum immunoglobulin levels, autoantibodies for autoimmune hepatitis and primary biliary cholangitis as well as a screening panel for Hepatitis B and C (HbsAg and anti- HCV with follow-on PCR if positive) and Hemoglobin A1c to test for type 2 diabetes mellitus. In addition, the patient should be evaluated for any component of the metabolic syndrome (arterial hypertension, central obesity and dyslipidemia) as Non-Alcoholic Fatty Liver Disease (NAFLD) is considered

its hepatic component. If any test of the liver etiology screening panel or the ultrasound is "positive" then the patient should be managed accordingly either locally or in a specialist center. In case the liver etiology panel is negative but the ultrasound suggests hepatic steatosis, the patient is in high risk of suffering from NAFLD. In this case, it is recommended that a first-line, non-invasive assessment of liver fibrosis, such as Fibrosis-4 (FIB-4) or NAFLD Fibrosis Score (NFS), is undertaken to identify patients with advanced fibrosis [7,8]. Patients with low FIB-4 (<1.3) or low NFS (<-1.455) can be managed in primary care and should be encouraged to make lifestyle changes in order to lose weight. Patients with intermediate FIB-4 (1.3-3.25) or NFS (-1.455-0.675) should undergo further testing with transient elastography (Fibroscan®) or ARFI elastography. Referral should be considered for patients with Fibroscan values >7.8 kPa along with patients with elevated FIB-4 (>3.25) and NFS (>0.675). Lastly, if all the results come back negative, then the physician should follow the patient and refer him/her to a specialist center for further diagnostic investigation in case of persistently abnormal results.

In case that the cholestatic enzymes (ALP and GGT) are elevated, an upper abdomen ultrasonography and a liver etiology screening panel should be ordered. The "screening panel" should definitely include serum immunoglobulin levels and autoantibodies for autoimmune liver diseases, namely autoimmune Hepatitis (Antinuclear Antibodies [ANA], Antibodies against Smooth Muscle [ASMA] and Liver/Kidney Microsome Antibodies [anti-LKM]) and primary biliary cholangitis (Anti-mitochondrial Antibodies [AMA]). Once there is an abnormal result, a referral should be made to a specialist center for management of the liver disease. If, on the other hand, the results come back negative, the cholestatic liver blood tests should be repeated. If still elevated, a referral should be again made so that further investigation may take place.

Finally, in the scenario that the liver blood tests are normal except for an isolated elevation of bilirubin, the physician should order some additional blood tests. Split bilirubin (unconjugated and conjugated) as well as a full blood count and lactate dehydrogenase (LDH) levels to test for hemolysis should be performed. If the tests for hemolysis are negative, the most probable diagnosis is Gilbert's syndrome which raises the unconjugated branch of bilirubin and is completely benign.

Liver disease's incidence is increasing in contrast to many other conditions, predominantly driven by the increasing prevalence of NAFLD. Elevation of liver tests is a common laboratory finding that is largely associated with liver diseases. Primary care physicians should be able to investigate the etiology of this finding and manage their patients accordingly. In this way, they will contribute substantially to the early diagnosis of liver diseases, thus halting their progression.

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare*

REFERENCES

1. Donnan PT, McLernon D, Dillon JF, Ryder S, Roderick P, Sullivan F et. al. Development of a decision support tool for primary care management of patients with abnormal liver function tests without clinically apparent liver disease: a record-linkage population cohort study and decision analysis (ALFIE). Health Technol Assess 2009;13:1–134.
2. Arndt V, Brenner H, Rothenbacher D, Zschenderlein B, Fraisse E, Fliedner TM. Elevated liver enzyme activity in construction workers: prevalence and impact on early retirement and all-cause mortality. Int Arch Occup Environ Health 1998;71(6):405-12.
3. Newsome PN, Cramb R, Davison SM, Dillon JF, Foulerton M, Godfrey EM, et al. Guidelines on the management of abnormal liver blood tests. Gut 2018; 67(1):6-19.
4. Kwo PY, Cohen SM, Lim JK. ACG Clinical Guideline. Evaluation of abnormal liver chemistries. Am J Gastroenterol 2017;112(1):18-35.
5. Dillon J, Miller MH, Robinson EM, Hapca A, Rezaeihemami M, Weatherburn C et. al. Intelligent Liver Function Testing (iLFT): A trial of automated diagnosis and staging of liver disease in Primary Care. J Hepatol. 2019;71(4):699-706.
6. Bush K, Kivlahan DR, McDonell MB, Fihn SD, Bradley KA. The AUDIT alcohol consumption questions (AUDIT-C): an effective brief screening test for problem drinking. Ambulatory Care Quality Improvement Project (ACQUIP). Alcohol Use Disorders Identification Test. Arch Intern Med. 1998;158(16):1789-95.
7. Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J et. al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006;43:1317-1325.
8. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC et. al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45(4):846-854.

Corresponding author:

Emmanouil K Sinakos
Assistant Professor of Internal Medicine and Hepatology,
4th Department of Internal Medicine, Aristotle University
of Thessaloniki, General Hospital of Thessaloniki "Ippokratio"
Tel.: +30 2310 892683,
E-mail: esinakos@auth.gr

ACHAIKI IATRIKI | 2021; 40(1):16–18

Management of acute pancreatitis according to guidelines: Are we compliant enough?

Christos Konstantakis, Georgia Diamantopoulou, Konstantinos Thomopoulos

Acute pancreatitis is an inflammatory process involving the pancreas, characterized by local inflammation and destruction of the pancreas due to activation of pancreatic enzymes followed in more severe cases by a systemic inflammatory response. Pancreatitis should be considered early in the differential diagnosis of acute abdomen, as it comprises 3% of the total cases. Despite recent advances in treatment, it remains a serious condition with a mortality rate of 3-8% [1,2].

Acute pancreatitis may vary from mild to severe with devastating course depending on the extent of local and systemic inflammation and complications. Based on Atlanta's latest classification, we distinguish three categories with different outcomes:

- Mild acute pancreatitis (80% of cases) is characterized by interstitial pancreatic and peripancreatic tissues edema without apparent tissue necrosis. There is no organ failure and no local or systemic complications and is usually self-limited.
- Moderately severe acute pancreatitis where we have transient (less than 48 hours) organ (s) failure or local complications.
- Severe acute pancreatitis which is characterized by organ(s) failure for more than 48 hours and / or local complications (pancreatic necrosis, abscess, pseudocyst). It is accompanied by high mortality (20-30%). In these cases, early diagnosis and aggressive management is mandatory [3].

Many predictive models have been proposed in the literature to predict the severity of acute pancreatitis based upon clinical, laboratory, and radiologic criteria. However, these models have neither high specificity nor sensitivity. Close patient monitoring remains of paramount importance.

Currently, there is no specific treatment for acute pancreatitis. Several different management algorithms have been recommended over the years. Supportive care with fluid resuscitation and pain control remain the main steps in the initial management of these patients [4-6]. Oral intake is initially discontinued (to put the pancreas at rest by reducing pancreatic exocrine secretion), while strong pain control is usually achieved by potent analgesics (e.g. pethidine) only.

Correction of hypovolemia and replenishment of fluids and electrolytes are of outmost importance. Patients with acute pancreatitis suffer from (mild to severe) loss of fluid in the third space, so in the early hours it is imperative to administer adequate fluids to the patient to compensate for this loss and maintain blood supply to vital organs [7,8]. Ringers crystalloid solutions are preferred [9]. Usually more than 3-4 liters in the first 24 hours are required. Caution should be exercised both in the elderly and in patients with coexisting (cardiac / renal) conditions to avoid overload. It is recommended to administer 20 ml / kg body weight for the first hour followed by 2 ml / kg / h intravenous infusion for the first 24 hours with close monitoring of vital signs, hematocrit and renal function.

We closely monitor the patient for other complications, especially hypoxia as the majority of deaths are related to respiratory failure. Mechanical support might be required in cases of acute respiratory distress syndrome. Placement of a nasogastric catheter which used to be the standard of care in the past, is now reserved only for cases of intestinal obstruction with persistent vomiting.

Department of Gastroenterology, University Hospital of Patras, Patras, Greece

Received: 27 Feb 2020; Accepted: 05 Mar 2020

Key words: *Pancreatitis; guidelines; management; complications*

Use of antibiotics in acute pancreatitis: Patients with acute pancreatitis have an increased risk of developing infections both local and systemic. Up to 20% of patients may develop extra-pancreatic (respiratory, urinary, etc.) infections that should be treated promptly and appropriately. In mild pancreatitis, prophylactic administration of antibiotics should be avoided. In severe cases, prophylactic use of antibiotics is still a topic of dispute. There is no strong evidence that the use of broad-spectrum antibiotics reduces the rate of septic complications or improves the overall clinical outcome of the disease. Therefore, treatment of patients should follow the "rules" of good clinical practice (i.e. aseptic handling) and appropriate antibiotic treatment should be reserved for those with valid clinical suspicion of infection [4,5,10].

Usually septic necrosis in severe pancreatitis occurs after the first week and is mainly due to the translocation of germs from the gut (Escherichia coli, Pseudomonas, Klebsiella and Enterococcus). In the event of infection (fever, no improvement or clinical deterioration, leukocytosis) broad-spectrum antibiotics with proven tissue penetration (carbapenem or quinolone, ceftazidime with metronidazole) should be administered. Blood cultures as well as fluid cultures from percutaneous aspiration of collections and necrotic areas provide significant assistance and should guide therapy.

Endoscopic retrograde cholangio - pancreatography (ERCP) in acute pancreatitis: In cases of stone impaction in the ampulla of Vater followed by persistent increase in bilirubin and / or coexistent cholangitis emergency ERCP with sphincterotomy and clearance of the bile duct is required. In cases of acute biliary pancreatitis without jaundice or cholangitis, even if there is suspicion of bile duct stone(s), ERCP should be postponed [1].

Nutrition in acute pancreatitis: In patients with acute pancreatitis, it is necessary to resume feeding no later than the second week after admission to avoid over catabolism. Oral feeding should be initiated as early as possible – as soon as it is tolerated (usually after pain is decreasing and inflammatory markers are improving). In those cases of (severe) pancreatitis where it is impossible to initiate oral feeding, artificial nutrition support is required [11,12]. Enteral feeding is preferred over parenteral nutrition, by administration of prepared solutions through a nasojejunal catheter or even through a nasogastric catheter. Intestinal nutrition preserves the integrity of the intestinal mucosa and reduces bacterial translocation and the risk of septic complications followed by intravenous administration [13].

Drainage of fluid collections, aseptic or septic necrosis: In cases of local complications that do not resolve spontaneously or cases of septic complication, it is necessary to aspirate and drain the fluid / necrotic collections. The aspirate is then examined (culture). Preferably, percutaneous and / or endoscopic drainage and debridement of necrotic collections is attempted. Surgical management is usually reserved for cases of failure of conservative treatment. In up to 65% of cases, surgery can be avoided by following a step-up approach [14]. Usually we prefer the endoscopic drainage of collections, where that is possible, transgastrically or transduodenally, and then double pig tail stents are inserted to create a cysto-gastrostomy or cysto-duodenostomy. In recent years, use of endoscopic ultrasound (EUS) and of metallic lumen apposing stents have facilitated fluid drainage and easier removal of infected and necrotic material respectively (Figure 1) [15].

The presence of infected pancreatic necrosis and/or endoscopic/radiologic failure are indications for surgery which ideally should be performed after the 3rd – 4th week of disease onset to allow the affected area to become walled off.

Cholecystectomy in biliary pancreatitis: Finally, in cases of gallstone acute pancreatitis, cholecystectomy is indicated as soon as possible. Following mild pancreatitis, performing laparoscopic cholecystectomy before the patient's discharge from the hospital seems to be both possible and beneficial because it reduces the rates of recurrence [16,17].

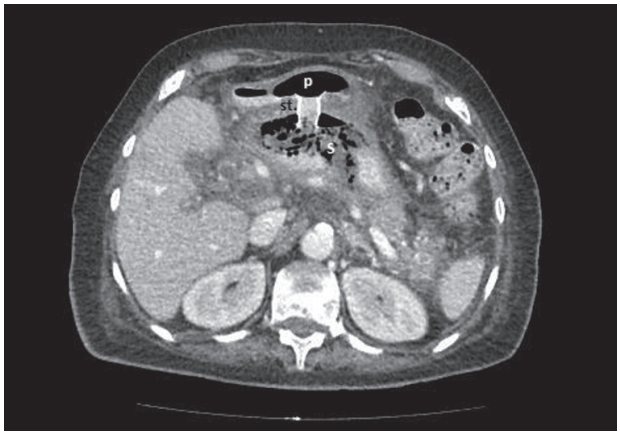


Figure 1. Drainage of the contents of a pancreatic pseudocyst into the stomach with the use of a metallic stent. P = pseudocyst S = stomach st. = stent.

In previous studies, lack of adherence to the published guidelines has been reported worldwide [18-20]. Compliance with recommendations for administration of fluids, antibiotics, nutritional support as well as timing of biliary interventions and surgery was not high enough. Overuse of antibiotics for inappropriate indications such us fever or infection prophylaxis has been reported as well as total parental nutrition instead of enteral nutrition especially by non-academic physicians [19]. In another recent study from Canada, 30.3% of patients with acute pancreatitis received prophylactic antibiotics and only 22.6% with gallstone pancreatitis underwent index admission cholecystectomy despite the existing evidence [20]. There is no data on the adherence to published guidelines by physicians in Greece, but it must be pointed out that strict follow-up and treatment of patients with acute pancreatitis according to published recommendations may reduce morbidity and mortality of these patients.

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare*

REFERENCES

1. Vege S, DiMagno M, Forsmark C, Martel M, Barkun A. Initial Medical Treatment of Acute Pancreatitis: American Gastroenterological Association Institute Technical Review. Gastroenterology. 2018;154(4):1103.
2. Van Santvoort H, Bakker O, Bollen T, Besselink M, Ahmed Ali U, Schrijver A, et al. A conservative and minimally invasive approach to necrotizing pancreatitis improves outcome. Gastroenterology. 2011;141(4):1254-63.
3. Banks P, Bollen T, Dervenis C, Gooszen H, Johnson C, Sarr M, Tsiotos G, Vege S, Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Acute Pancreatitis Classification Working Group Gut. 2013;62(1):102.
4. Working Group IAP/APA Acute Pancreatitis Guidelines. IAP/APA evidence-based guidelines for the management of acute pancreatitis. Pancreatology. 2013;13(4 Suppl 2):e1-15.
5. Crockett S, Wani S, Gardner T, Falck-Ytter Y, Barkun A, American Gastroenterological Association Institute Clinical Guidelines Committee American Gastroenterological Association Institute Guideline on Initial Management of Acute Pancreatitis. Gastroenterology. 2018;154(4):1096.
6. Pezzilli R, Zerbi A, Campra D, Capurso G, Golfieri R, et al. Italian Association for the Study of the Pancreas (AISP). Consensus guidelines on severe acute pancreatitis. Dig Liver Dis. 2015;47(7):532-43.
7. Trikudanathan G, Navaneethan U, Vege SS. Current controversies in fluid resuscitation in acute pancreatitis: a systematic review. Pancreas. 2012; 41:827.
8. Gardner T, Vege S, Chari S, Petersen B, Topazian M, Clain JE, et al. Faster rate of initial fluid resuscitation in severe acute pancreatitis diminishes in-hospital mortality. Pancreatology. 2009;9:770.
9. Wu B, Hwang J, Gardner T, Repas K, Delee R, Yu S, et al. Lactated Ringer's solution reduces systemic inflammation compared with saline in patients with acute pancreatitis. Clin Gastroenterol Hepatol. 2011;9:710.
10. Tenner S, Baillie J, DeWitt J, Vege S. American College of Gastroenterology guideline: management of acute pancreatitis. Am J Gastroenterol 2013; 108:1400.
11. McClave S, Chang W, Dhaliwal R, Heyland D. Nutrition support in acute pancreatitis: a systematic review of the literature. JPEN J Parenter Enteral Nutr. 2006;30:143.
12. Petrov M, Pylypchuk R, Emelyanov N. Systematic review: nutritional support in acute pancreatitis. Aliment Pharmacol Ther. 2008;28:704.
13. Al-Omran M, Albalawi Z, Tashkandi M, Al-Ansary L. Enteral versus parenteral nutrition for acute pancreatitis. Cochrane Database Syst Rev. 2010; CD002837.
14. Van Santvoort H, Besselink M, Bakker O, Hofker H, Boermeester M, Dejong C, et al. A step-up approach or open necrosectomy for necrotizing pancreatitis. N Engl J Med. 2010;362(16):1491-502.
15. Lang G, Fritz C, Bhat T, Das K, Murad F, Early D, et al. EUS-guided drainage of peripancreatic fluid collections with lumen-apposing metal stents and plastic double-pigtail stents: comparison of efficacy and adverse event rates. Gastrointest Endosc. 2018;87(1):150-157.
16. Aboulian A, Chan T, Yaghoubian A, Kaji A, Putnam B, Neville A, et al. Early cholecystectomy safely decreases hospital stay in patients with mild gallstone pancreatitis: a randomized prospective study. Ann Surg. 2010;251(4):615-9.
17. Da Costa D, Bouwense S, Schepers N, Besselink M, van Santvoort H, van Brunschot S, et al. Same-admission versus interval cholecystectomy for mild gallstone pancreatitis (PONCHO): a multicentre randomised controlled trial. Lancet. 2015;386(10000):1261-1268.
18. Pezzilli R, Uomo G, Gabbrielli A, Zerbi A, Frulloni L, De Rai P, et al. A prospective multicentre survey onthe treatment of acute pancreatitis in Italy. Dig Liver Dis. 2007;39(9):838-46.
19. Sun E, Tharakan M, Kapoor S, Chakravarty R, Salhab A, Buscaglia J, et al. Poor compliance with ACG guidelines for nutrition and antibiotics in the management of acute pancreatitis: A North American survey of gastrointestinal specialists and primary care physicians. JOP. 2013;14(3):221-7.
20. Greenberg J, Hsu J, Bawazeer M, Marshall J, Friedrich J, Nathens A, et al. Compliance with Evidence-Based Guidelines in Acute Pancreatitis: an Audit of Practices in University of Toronto Hospitals. J Gastrointest Surg. 2016;20(2):392-400.

Corresponding author:

Christos Konstantakis
MD University Hospital of Patras, D. Stamatopoulou 4,
Rio 26504, Patras, Greece
Tel.: +30 6974 563157, Fax: +30 261 0999518,
E-mail: asraiah@yahoo.com

Patient Reported Outcomes

Karolina Akinosoglou, Charalambos Gogos

INTRODUCTION

Although traditionally, morbidity or mortality has been the main criterion of a therapy's efficacy, quantification of assessment of disease outcomes from a patient's point of view has been increasing during the last decades. A new therapy can show excellent results in the improvement of respective biomarkers involved, or even prolong life expectancy in the context of clinical trials, however, in real life patients may indicate they could not adhere to treatment due to side effects, complex dosing schemes, and reduced quality of life. Similarly, therapists seem to commonly underestimate the severity of the disease or be unable to describe the full range of clinical manifestations - the patient is experiencing [1]. The impact of a disease on patients' quality of life cannot be predicted, and by no means can be correlated with the severity of the disease itself from a medical point of view. As a result, the use of information reported by patients (patient reported outcomes - PRO) to further evaluate treatment outcomes by health professionals, is increasingly gaining ground as a way to understand and improve the quality of healthcare services [2].

A PRO is defined as modification or interpretation of the response from a health professional, or any other" [3]. The tools/instruments by which PROs are evaluated and recorded, capturing patients' perception regarding their state of health, disease and the effects of therapeutic interventions, are called PRO measures (PROMs). Many PROMs are designed to be used in a variety of diseases or, may be specific to a particular disease or population. In clinical practice, PROs can be used by patients in a self-report format, to detect various clinical manifestations that otherwise may go undetected. In addition, they can be used for monitoring treatment efficacy that common surrogate biomarkers or available indices cannot assess. Lastly, they allow patients' more active participation to their management plan, that ultimately promotes a more human-centered healthcare [4]. However, PRO should not be confused with the recording of disease symptoms from their attending physicians. These patient self-report outcomes represent a much more multi-dimensional entity, driven entirely by the patient.

PROM DESIGN

PROs have many dimensions, that can be measured with the right tools. These tools usually consist of self-completion questionnaires, which may be generic, e.g. EQ-5D (Euro Quality of life - 5 Dimension) examining quality of life, or disease-specific e.g. SGRQ (St'George's Respiratory Questionnaire) for chronic obstructive pulmonary disease [5]. Even though, PROMs are designed by the scientific community, involvement of the respective patient community that is addressed by each PROM is pivotal, before, during, and after the design and implementation of such a tool. Patients are the most suitable to develop, evaluate and review tools, recognize the need for new ones, supply with new study objects depending on community needs, and finally adopt them .

The development and evaluation of PROMs is an ongoing and detailed process that comprises content validity, reliability and responsiveness [5, 6]. Briefly, once the concept of interest is defined, patients are asked questions about this. These questions are defined as "objects". The objects are queried to determine the variation of a concept. Main concepts evaluated in PROM include quality of life, degree of patient's satisfaction, physical and social functioning, psychological state, signs and symptoms, compliance with treatment etc. Objects must be clear, valid and their number depends

Department of Internal Medicine and Infectious Diseases, University General Hospital of Patras, 26504, Rio, Patras, Greece

Received: 14 Sep 2020; Accepted: 23 Dec 2020

Key words: *Patient reported outcomes; patient reported outcome measures*

on the completeness of information they provide. They are continuously reassessed and improved based on new needs, and must be adapted to the target-population and individual level of cognitive function and linguistic preference. Each object should at the same time ensure a reasonable recall period (to what extent in the past patients are called to answer information about their condition), and adequate options of response (graphic, quantitative scale, verbal) [7]. Of note, the patient's response to the same question can be different, depending on the time of day he is called to answer, on the type of distribution of the questionnaire (electronic, telephone etc), and the results may vary depending on the questionnaire completion instructions and patient's literacy level. Clearly, the purpose of the tool, the characteristics of the disease, the duration and frequency of symptoms, the purpose and intended use of PROM determine objects. Before their final configuration, the tools are tested by patients and experts and respectively corrected if, for example, objects are not clear or relevant to the concept (not considered relevant, requiring frequent clarification), if a limited response range is noted (preventing answer variation), have little diversity (all patients give the same answer), if there is little change detection sensitivity, or redundancy is observed etc. Reliability i.e. the measurements are repeatable and stable, and distinguish between changes in the response, and validity i.e tool measures what it is intended to measure and what is important for patients, are important to structure a useful PROM.

PRO APPLICATIONS AND CHALLENGES

Initially, PROs were developed primarily for use in pharmacological and medical research [8]. However, now PRO tools seem to extend far beyond clinical research, given their ability to transform the healthcare system and improve the quality of services by placing the patient at the center of decisions. Besides assessing parameters that may not be measured by the treating physician, and complement information available from existing and measurable bio-markers, they seem to have predictive value in survival [9] and hospitalization rates [10]. Thus, they can be used from all parties involved, including patients, healthcare givers, insurance companies, healthcare systems to evaluate quality of care and reform budgets. These changes have made regulatory agencies such as the FDA (US Food and Drug Administration) and the EMA (European Medicine Agency), to recommend their use in the evaluation and promotion of new therapeutic interventions and further optimize them, as seen in a variety of products of different disciplines [11]. However, their implementation is far from an easy task. The heterogeneity of tools, the timing of the various stages of the disease that the tools are distributed, the current illiteracy levels, or the exacerbation of anxiety the tools themselves create, remain just some of the challenges [12]. After all, no one-size PRO fits all kinds of patients.

Key points

- Clinical trial endpoints often fail to be translated to meaningful clinical outcomes for patients involved.
- Patient Reported Outcomes represent patients' report of their state of health, without correction, modification, or interpretation of the response from a health professional, or any other.
- Patient Reported Outcomes can be assessed by specifically designed and constructed tools, i.e. Patient Reported Outcome Measures.
- Patient Reported Outcomes find application from clinical research, to every-day clinical practice and improvement of healthcare policies.

Conflict of interest disclosure: *KA has received research, advisory and honorary grants from Gilead Sciences, Angelini, Pfizer Hellas, CG has received research, advisory and honorary grants from Gilead Sciences, Pfizer Hellas, Astellas, Merck Sharp and Dohme and GlaxoSmithKline.*

Declaration of funding sources: *Data for this manuscript were collected within the context and in support of a project for the production of non-promotional, non-product related, educational material on PROs/PROMs in HIV infection (University of Patras and Gilead Sciences Greece).*

REFERENCES

1. Chassany O, Le-Jeunne P, Duracinsky M, Schwalm MS, Mathieu M. Discrepancies between patient-reported outcomes and clinician-reported outcomes in chronic venous disease, irritable bowel syndrome, and peripheral arterial occlusive disease. Value Health. 2006;9(1):39-46.
2. Boyce MB, Browne JP. Does providing feedback on patient-reported outcomes to healthcare professionals result in better outcomes for patients? A systematic review. Qual Life Res. 2013;22(9):2265-78.
3. US Food and Drug Administration. Guidance for industry. Patient-reported outcomes measures: use in medical product development to support labelling claims. FDA PRO Guidance2009. Available from: https://www.fda.gov/

regulatory-information/search-fda-guidance-documents/patient-reported-outcome-measures-use-medical-product-development-support-labeling-claims
4. Santana MJ, Feeny D. Framework to assess the effects of using patient-reported outcome measures in chronic care management. Qual Life Res. 2014;23(5):1505-13.
5. Weldring T, Smith SM. Patient-Reported Outcomes (PROs) and Patient-Reported Outcome Measures (PROMs). Health Serv Insights. 2013;6:61-8.
6. Rothrock NE, Kaiser KA, Cella D. Developing a valid patient-reported outcome measure. Clin Pharmacol Ther. 2011;90(5):737-42.
7. Deshpande PR, Rajan S, Sudeepthi BL, Abdul Nazir CP. Patient-reported outcomes: A new era in clinical research. Perspect Clin Res. 2011;2(4):137-44.
8. Mercieca-Bebber R, King MT, Calvert MJ, Stockler MR, Friedlander M. The importance of patient-reported outcomes in clinical trials and strategies for future optimization. Patient Relat Outcome Meas. 2018;9:353-67.
9. Pinheiro LC, Zagar TM, Reeve BB. The prognostic value of pre-diagnosis health-related quality of life on survival: a prospective cohort study of older Americans with lung cancer. Qual Life Res. 2017;26(7):1703-12.
10. Emuren L, Welles S, Polansky M, Evans AA, Macalino G, Agan BK, et al. Lower health-related quality of life predicts all-cause hospitalization among HIV-infected individuals. Health Qual Life Outcomes. 2018;16(1):107.
11. Gnanasakthy A, Mordin M, Evans E, Doward L, DeMuro C. A Review of Patient-Reported Outcome Labeling in the United States (2011-2015). Value Health. 2017;20(3):420-9.
12. Boyce MB, Browne JP, Greenhalgh J. The experiences of professionals with using information from patient-reported outcome measures to improve the quality of healthcare: a systematic review of qualitative research. BMJ Qual Saf. 2014;23(6):508-18.

Corresponding author:

K. Akinosoglou
Department of Internal Medicine and Infectious Diseases,
University General Hospital of Patras, Greece,
Tel: +30 2610 999582
E-mail: akin@upatras.gr

New advances in the management of differentiated thyroid cancer

Georgios K. Markantes, Marina A. Michalaki

Thyroid cancer (TC) accounts for 88% of all endocrine carcinomas [1], is the fifth commonest new cancer diagnosis in women and the eighth most common new cancer diagnosis overall in the US [2]. Differentiated thyroid carcinoma (DTC), namely papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), constitutes 85-95% of all TC cases [3,4]. PTC represents 90-95% of DTC [3] and is considered an indolent tumor with 10- and 30-year disease-specific mortality rates less than 5% [5] and 10% [6], respectively. Rare and more aggressive subtypes of TC also exist, such as poorly differentiated thyroid cancers (6% of TC), anaplastic carcinoma (<1% of TC) and the C-cell origin medullary carcinoma-MTC (4% of TC) [3]. Over the last two decades, a rising incidence of TC is observed worldwide that can be attributed almost entirely to PTC [7-9]. The observed universal rise in PTC incidence rates is probably due to the increased sensitivity of modern diagnostic methods, since only the incidence of small (T1) PTC increases, while mortality rates remain stable or even decrease [10]. The widespread use of thyroid ultrasonography has led to an increased incidental diagnosis of TC in asymptomatic individuals [8,11].

For many years, the standard of care for all DTC patients included total thyroidectomy with or without cervical lymph node dissection, routine post-operative radioactive I131 (RAI) therapy and thyroid hormone suppressive therapy. After initial treatment, DTC patients were closely followed for life, since recurrences or death could occur even thirty years after the initial diagnosis [6]. However, several clinical trials have shown that such an approach is unnecessarily aggressive for the majority of DTC patients. Therefore, the recently developed guidelines from the American Thyroid Association (ATA) [12] propose a less aggressive initial approach and a less intensive follow up for selected DTC patients.

In particular, they suggest that lobectomy could replace total thyroidectomy in intrathyroidal DTC tumors measuring less than 4cm. Suppressive therapy with levothyroxine which causes iatrogenic subclinical hyperthyroidism with well recognized deleterious health consequences especially in the elderly and in postmenopausal women, is reserved only for patients with metastatic disease or at high risk for recurrence. Furthermore, they propose a more conservative use of postoperative RAI therapy with limited indications, lower activities (as low as 30mCi) and without thyroid hormone withdrawal but with the newly developed human recombinant thyrotropin (rhTSH). Depending on the primary goal of postoperative RAI administration, ATA classifies RAI therapy as: "RAI ablation therapy", intended to destroy benign thyroid remnants and facilitate follow up; "RAI adjuvant therapy", aiming to destroy suspected but unproven residual disease and decrease recurrences; and "RAI treatment", designated to destroy residual or metastatic disease and improve survival [12]. In this guideline, it is also acknowledged that DTC has a distinct biological behavior and though death is rare, recurrences or persistent disease could be more frequent. Therefore, besides the classical AJCC/UICC/TNM staging system which predicts mortality, the ATA designed another staging system to evaluate the risk of recurrence and/or persistent disease in DTC patients. The 2015 ATA risk stratification system stratifies patients into three risk groups (low, intermediate, and high) for

Division of Endocrinology, Department of Internal Medicine, University of Patras, School of Health Sciences, Patras, Greece

Received: 05 May 2020; Accepted: 29 Jun 2020

Key words: *Thyroid cancer; differentiated thyroid cancer; management; RAI; tyrosine kinase inhibitors*

recurrent/persistent disease, depending on their clinicopathological characteristics after thyroidectomy [12]. The usefulness of this system lies in its availability to aid clinicians in determining the need for RAI therapy after thyroidectomy, the degree of TSH suppression and the intensity of follow-up. Accordingly, RAI ablation therapy after thyroidectomy is not routinely recommended for unifocal or multifocal papillary tumors <1cm, neither for other ATA low-risk DTC patients in the absence of any other adverse feature. Moreover, RAI adjuvant therapy is not recommended but considered in patients at intermediate risk for recurrence or persistent disease [12].

However, other scientific societies have questioned many recommendations of the 2015 ATA guidance. More specifically, the European Thyroid Association (ETA) disagreed with the proposed extent of surgery (lobectomy) for low-risk DTC tumors >2 and <4cm. They stated three reasons. Firstly, in many European countries with current or recent longstanding iodine deficiency, multinodular disease and bilateral involvement are frequent; secondly, a significant number of patients undergoing lobectomy (30-43%) will need completion total thyroidectomy due to the discovery of high-risk features in pathology reports; finally, lobectomy precludes the subsequent use of RAI therapy [13]. Besides, the ETA, the European Association of Nuclear Medicine (EANM) and the Society of Nuclear Medicine and Molecular Imaging (SNMMI) had many concerns regarding the optimal selection of patients for RAI therapy postoperatively as well as the recommended I131 activities for adjuvant therapy [13-15]. European experts favor retaining the longstanding, widely applied practice of RAI postoperative therapy in low- and intermediate-risk patients, since data from prospective, controlled, randomized trials are currently lacking [13]. Notably, two ongoing prospective, multicenter, RCTs -IoN from the United Kingdom and ESTIMABL 2 from France (clinicaltrials.gov identifiers NCT01398085 and NCT01837745, respectively)- are comparing I131 in a dose of 1.11 GBq (30mCi) versus no I131 in low-risk and, in the case of IoN, also in intermediate-risk patients.

Finally, during the last decade, much progress has been made in understanding the molecular landscape of thyroid cancer. Activating mutations of genes encoding effectors of the mitogen-activated protein kinase (MAPK) and PI3/AKT/mTOR pathways have been implicated in the pathogenesis of DTC [3]. These two classical downstream pathways are coupled with receptors with tyrosine kinase activity. BRAFV600E is the most common driver mutation occurring in approximately 50-60% of PTC and rather predisposing to aggressive tumor behavior, while RAS mutations account for 15% of FTCs [3]. Chromosomal rearrangements of several receptors with tyrosine kinase activity, such as RET, NTRK, and ALK have also been described in DTC [3]. Advances in understanding the molecular profile of these tumors lead to improvements in the diagnosis and treatment of DTC patients. Although DTC is considered a tumor with excellent prognosis, approximately 30% of patients with distant metastatic disease do not respond to RAI therapy [16]. "RAI refractory" patients have an unfavorable prognosis with overall survival of <50% at 3 years [5]. Sorafenib and lenvatinib are inhibitors of receptors with tyrosine kinase activity (TKI) and recently they have been used for the treatment of DTC patients with advanced, progressing and RAI refractory disease. However, despite increasing the progression-free survival, they do not increase overall survival, and have numerous adverse effects that severely impair patients' quality of life [3]. RAI therapy in DTC is based on the ability of thyrocytes to accumulate RAI via the Na+/I- symporter (NIS). RAI-refractory disease develops because NIS expression is suppressed or even absent in a subgroup of malignant thyroid tumors. Novel TKIs (such as selumetinib) that can restore NIS expression and consequently RAI uptake with satisfactory clinical outcomes have recently been developed. All these drugs are currently being tested in clinical trials [16].

In conclusion, the current approach of DTC patients is more sophisticated, less aggressive, and more cost-effective than in the recent past. However, it is surprising that throughout the last century, the mortality and morbidity rates of DTC have practically remained unaltered [17]. The development of novel, targeted therapies based on molecular data will mark the beginning of a new, very promising era in the management of DTC.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *None to declare.*

REFERENCES

1. Van der Zwan JM, Mallone S, Van Dijk B, Bielska-Lasota M, Otter R, Foschi R, et al. Carcinoma of endocrine organs: results of the RARECARE project. Eur J Cancer. 2012;48:1923-31.
2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin. Jan-Feb 2014;64(1):9-29.
3. Fagin JA, Wells SA Jr. Biologic and Clinical Perspectives on Thyroid Cancer. N Engl J Med. 2016;375(23):1054-67.

4. Sherman SI. Thyroid carcinoma. Lancet. 2003;361(9356):501–11.
5. Riesco-Eizaguirre G, Santisteban P. ENDOCRINE TUMOURS: Advances in the Molecular Pathogenesis of Thyroid Cancer: Lessons From the Cancer Genome. Eur J Endocrinol. 2016;175(5):R203-17.
6. Dong W, Horiuchi K, Tokumitsu H, Sakamoto A, Noguchi E, Ueda Y, et al. Time-Varying Pattern of Mortality and Recurrence From Papillary Thyroid Cancer: Lessons From a Long-Term Follow-Up. Thyroid. 2019;29(6):802-8.
7. Wiltshire J, Drake T, Uttley L, Balasubramanian B. Systematic Review of Trends in the Incidence Rates of Thyroid Cancer. Thyroid. 2016;26(11):1541-52.
8. Davies L, Welch HG. Increasing incidence of thyroid cancer in the United States, 1973-2002. JAMA. 2006;295(18):2164-67.
9. Pellegriti G, Frasca F, Regalbuto C, Squatrito S, Vigneri R. Worldwide increasing incidence of thyroid cancer: update on epidemiology and risk factors. J Cancer Epidemiol. 2013; 2013:965212.
10. Li M, Brito JP, Vaccarella S. Long-Term Declines of Thyroid Cancer Mortality: An International Age–Period–Cohort Analysis. Thyroid. 2020;30(6):838-846.
11. Russ G, Leboulleux S, Leenhardt L, Hegedüs L. Thyroid incidentalomas: epidemiology, risk stratification with ultrasound and workup. Eur Thyroid J. 2014;3(3):154-63.
12. Haugen BR, Alexander EK, Bible KC, Doherty GM, Mandel SJ, Nikiforov YE, et al. 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. Thyroid. 2016;26(1):1-133.
13. Luster M, Aktolun C, Amendoeira I, Barczyński M, Bible KC, Duntas LH, et al. European perspective on 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer. Proceedings of an interactive international symposium. Thyroid. 2019;29(1):7-26.
14. Verburg FA, Aktolun C, Chiti A, Frangos S, Giovanella L, Hoffmann M, et al. Why the European Association of Nuclear Medicine has declined to endorse the 2015 American Thyroid Association management guidelines for adult patients with thyroid nodules and differentiated thyroid cancer. Eur J Nucl Med Mol Imaging. 2016;43(6):1001–05.
15. Tuttle RM, Ahuja S, Avram AM, Bernet VJ, Bourguet P, Daniels GH, et al. Controversies, Consensus, and Collaboration in the Use of 131 I Therapy in Differentiated Thyroid Cancer: A Joint Statement From the American Thyroid Association, the European Association of Nuclear Medicine, the Society of Nuclear Medicine and Molecular Imaging, and the European Thyroid Association. Thyroid. 2019;29(4):461-70.
16. Jhiang SM, Konda B, Sipos JA, Nabhan FA. Prospects for Redifferentiating Agents in the Use of Radioactive Iodine Therapy for Thyroid Cancer. Thyroid. 2020;30(4):471-73.
17. McConahey WM, Hay ID, Woolner LB, van Heerden JA, Taylor WF. Papillary Thyroid Cancer Treated at the Mayo Clinic, 1946 Through 1970: Initial Manifestations, Pathologic Findings, Therapy, and Outcome. Mayo Clin Proc. 1986;61(12):978-96.

Corresponding author:

Marina A. Michalaki
Endocrinology, Department of Internal Medicine, University of Patras School of Health Sciences, Patras, PC 26500, Greece
Tel: +30 2610 999427
E-mail: mixmar@upatras.gr

What is new in endometrial cancer treatment?

Georgios Androutsopoulos

Endometrial cancer (EC) is the second most common malignancy of the female reproductive system worldwide [1]. It is more prevalent in developed regions (North America, Europe, Australia and New Zealand), compared with less developed ones (Central and South America, Asia and Africa) [1]. The disease usually affects postmenopausal women and the average annual incidence is about 2.1% [1]. Most patients have abnormal uterine bleeding, as the main presenting symptom [1].

Classification of sporadic EC in 2 different types based on clinical and pathological features, is widely acceptable and plays an important role in patient management [2]. These 2 types have distinct etiology, natural history and clinical behavior [2]. More specifically, type I EC is more common (70-80% of sporadic cases) and endometrioid in histology, has a less aggressive clinical behavior and a more favorable prognosis [2]. In contrast, type II EC is less common (10-20% of sporadic cases) and papillary serous, clear cell or undifferentiated in histology, has a more aggressive clinical behavior and a less favorable prognosis [2].

According to recently published guidelines, systematic surgical staging represents the initial therapeutic approach in EC patients [3-6]. Treatment planning should be made by a multidisciplinary team (MDT) including: gynecological oncologist, radiation oncologist, medical oncologist, pathologist and radiologist [6]. During the MDT meeting, all available treatment options should be considered and the extent of surgical procedure and the type of postoperative adjuvant treatment should be carefully individualized according to disease stage, histologic subtype, fertility issues and patient general status [3-6].

Gynaecological Oncology Unit, Department of Obstetrics and Gynaecology, University of Patras, Medical School, Rion, Greece

Received: 13 May 2020; Accepted: 02 Nov 2020

The systematic surgical staging in patients with EC includes: total hysterectomy, bilateral salpingo-oophorectomy, pelvic and para-aortic lymphadenectomy and complete resection of any suspicious lesion [3-6]. Especially, in patients with cervical stroma involvement, a modified radical hysterectomy should be performed in order to obtain clear margins [3-6]. Furthermore, in EC patients with a high risk disease (tumor grade III, nonendometrioid histology), total omentectomy is a necessary part of the systematic surgical staging procedure [3-6]. Although peritoneal washings have no effect on FIGO staging, they should be obtained in all EC cases as positive result represents an adverse risk factor [3-6].

All these systematic surgical staging procedures should be performed by a Gynaecologist with appropriate training in Gynaecological Oncology, using either the open (laparotomy) or the minimally invasive (laparoscopy and robotic-assisted surgery) surgical approach [3-6]. Both surgical approaches can be used in EC patients with early stage disease, as they provide similar recurrence, overall survival and disease-free survival rates [4-6]. However, in high risk EC patients as well as in patients with advanced stage disease, laparotomy is the standard surgical approach allowing complete surgical staging and aggressive cytoreduction [3-6].

Pelvic and para-aortic lymphadenectomy represents an essential part of systematic surgical staging in EC patients, as this is the only way to diagnose accurately FIGO stage IIIC disease [3-6]. The systematic pelvic lymph node dissection includes: removal of the lymphatic tissue from the distal half of the common iliac vessels, the external iliac vessels (down to the deep circumflex iliac

Key words: *endometrial cancer; surgery; radiotherapy; chemotherapy; treatment*

vein) and the obturator fossa (above to the obturator nerve) [3-6]. Likewise, the systematic para-aortic lymph node dissection includes: removal of the lymphatic tissue from the aorta and the inferior vena cava (up to the level of renal vessels or inferior mesenteric artery) [3-6].

Based on the SEPAL study findings, the systematic pelvic and para-aortic lymph node dissection should be routinely performed in intermediate and high risk EC patients (stage IB or more in type I EC and any stage in type II EC), as they offer survival benefits [7]. There is a direct correlation between the extent of the systematic pelvic and para-aortic lymphadenectomy and the risk for perioperative complications (vascular or nerve injury, lymphocyst, lymphoedema and cellulitis formation) [3, 8-10]. Especially, in elderly or obese patients with co-existing comorbidities (diabetes mellitus, coronary artery disease), there is a substantial increase in morbidity and perioperative complication rates, that should be carefully balanced with any survival advantage [3, 8-10].

According to the FIRES trial findings, sentinel lymph node mapping and dissection could be routinely performed in EC patients with early stage disease [11]. The main advantage of the sentinel lymph node technique, is the significant reduction of perioperative complications [3-6, 12]. Apart from identified sentinel lymph nodes, all enlarged or suspicious lymph nodes should also be removed based on the established surgical algorithm [12, 13]. In case of technique failure in one side, a side-specific systematic pelvic lymph node dissection should be performed [12]. All dissected sentinel lymph nodes should be evaluated with the ultra-staging approach [12].

In EC patients with early stage disease (stage I and II) and one or more adverse risk factors (age ≥60 years, depth of invasion >50%, tumor volume, lymphovascular space invasion, tumor grade III, nonendometrioid histology), postoperative adjuvant treatment (radiotherapy and/or systemic therapy) should be administered [3-6].

More specifically, in high-intermediate risk EC patients (age ≥60 years, depth of invasion >50%, lymphovascular space invasion) with early stage disease, postoperative adjuvant radiotherapy remains the treatment of choice based on findings of the GOG-99 and PORTEC-1 trials [3-6, 14, 15]. Adjuvant radiotherapy could be either vaginal brachytherapy and/or external pelvic radiotherapy and should be initiated no longer than 3 months after the systematic surgical staging [5]. Both radiotherapeutic approaches minimize the risk for local recurrences, but have no effect on overall and disease free survival (GOG-99, PORTEC-1 and PORTEC-2 trials) [14-16]. When comparing both of them, vaginal brachytherapy is more tolerated and associated with fewer side effects and better quality of life (PORTEC-2 trial) [16].

Moreover, in high risk EC patients (tumor grade III, nonendometrioid histology) with early stage disease, postoperative adjuvant radiotherapy could be possibly combined with postoperative adjuvant systemic therapy, based on the PORTEC-3 trial findings [17]. It seems that the combination of both therapeutic approaches in high risk EC patients with early stage disease, is associated with persistent sensory neurological symptoms and a minimal improvement in overall and disease free survival (GOG-249 and PORTEC-3 trial) [17, 18].

In EC patients with advanced stage disease (stage III and IV), postoperative adjuvant treatment (systemic therapy and/or radiotherapy) should also be administered [3-6].

More specifically, in EC patients with advanced stage disease, postoperative adjuvant systemic therapy represents the treatment of choice according to the GOG-122 trial findings [19]. Adjuvant multiagent systemic therapy (doxorubicin - cisplatin) significantly improves overall and disease free survival in EC patients with advanced stage disease, when compared with whole abdomen radiotherapy alone (GOG-122 trial) [19]. However, the administration of multiagent systemic therapy was associated with increased toxicity rates (GOG-122 trial) [19].

Moreover, in EC patients with advanced stage disease, postoperative adjuvant systemic therapy could be combined with adjuvant radiotherapy, based on the PORTEC-3 trial findings [17]. It seems that the combination of both therapeutic approaches in EC patients with stage III disease, is associated with significant improvement in overall and disease free survival, when compared with external radiotherapy alone (PORTEC-3 trial) [17]. Moreover, the combination of both therapeutic approaches in EC patients with stage III or IVA disease, does not provide any benefit regarding disease free survival, when compared with systemic therapy alone (GOG-258 trial) [20].

In conclusion, systematic surgical staging remains the initial therapeutic approach in EC patients [3-6]. In these cases, all available treatment options should be considered by a multidisciplinary team (MDT) and treatment planning should be carefully individualized based on disease stage, histologic subtype, fertility issues and patient performance status [3-6].

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *None to declare.*

REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2. Bokhman J. Two pathogenetic types of endometrial carcinoma. Gynecol Oncol. 1983;15(1):10-7.
3. ACOG. Practice Bulletin No. 149: Endometrial cancer. Obstet Gynecol. 2015;125(4):1006-26.
4. Colombo N, Creutzberg C, Amant F, Bosse T, Gonzalez-Martin A, Ledermann J, et al. ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer: diagnosis, treatment and follow-up. Ann Oncol. 2016;27(1):16-41.
5. Koh W, Abu-Rustum N, Bean S, Bradley K, Campos S, Cho K, et al. Uterine Neoplasms, Version 1.2018, NCCN Clinical Practice Guidelines in Oncology. Journal of the National Comprehensive Cancer Network: JNCCN. 2018;16(2):170-99.
6. Amant F, Mirza M, Koskas M, Creutzberg C. Cancer of the corpus uteri. Int J Gynaecol Obstet. 2018;143 Suppl 2:37-50.
7. Todo Y, Kato H, Kaneuchi M, Watari H, Takeda M, Sakuragi N. Survival effect of para-aortic lymphadenectomy in endometrial cancer (SEPAL study): a retrospective cohort analysis. Lancet. 2010;375(9721):1165-72.
8. Franchi M, Ghezzi F, Riva C, Miglierina M, Buttarelli M, Bolis P. Postoperative complications after pelvic lymphadenectomy for the surgical staging of endometrial cancer. J Surg Oncol. 2001;78(4):232-7; discussion 7-40.
9. Benedetti Panici P, Basile S, Maneschi F, Alberto Lissoni A, Signorelli M, Scambia G, et al. Systematic pelvic lymphadenectomy vs. no lymphadenectomy in early-stage endometrial carcinoma: randomized clinical trial. J Natl Cancer Inst. 2008;100(23):1707-16.
10. May K, Bryant A, Dickinson H, Kehoe S, Morrison J. Lymphadenectomy for the management of endometrial cancer. Cochrane Database Syst Rev. 2010;(1):CD007585.
11. Rossi E, Kowalski L, Scalici J, Cantrell L, Schuler K, Hanna R, et al. A comparison of sentinel lymph node biopsy to lymphadenectomy for endometrial cancer staging (FIRES trial): a multicentre, prospective, cohort study. Lancet Oncol. 2017;18(3):384-92.
12. Abu-Rustum NR. Sentinel lymph node mapping for endometrial cancer: a modern approach to surgical staging. Journal of the National Comprehensive Cancer Network: JNCCN. 2014;12(2):288-97.
13. Barlin JN, Khoury-Collado F, Kim C, Leitao M, Jr., Chi D, Sonoda Y, et al. The importance of applying a sentinel lymph node mapping algorithm in endometrial cancer staging: beyond removal of blue nodes. Gynecol Oncol. 2012;125(3):531-5.
14. Creutzberg C, van Putten W, Koper P, Lybeert M, Jobsen J, Warlam-Rodenhuis C, et al. Surgery and postoperative radiotherapy versus surgery alone for patients with stage-1 endometrial carcinoma: multicentre randomised trial. PORTEC Study Group. Post Operative Radiation Therapy in Endometrial Carcinoma. Lancet. 2000;355(9213):1404-11.
15. Keys H, Roberts J, Brunetto V, Zaino R, Spirtos N, Bloss J, et al. A phase III trial of surgery with or without adjunctive external pelvic radiation therapy in intermediate risk endometrial adenocarcinoma: a Gynecologic Oncology Group study. Gynecol Oncol. 2004;92(3):744-51.
16. Nout R, Smit V, Putter H, Jurgenliemk-Schulz I, Jobsen J, Lutgens L, et al. Vaginal brachytherapy versus pelvic external beam radiotherapy for patients with endometrial cancer of high-intermediate risk (PORTEC-2): an open-label, non-inferiority, randomised trial. Lancet. 2010;375(9717):816-23.
17. de Boer S, Powell M, Mileshkin L, Katsaros D, Bessette P, Haie-Meder C, et al. Adjuvant chemoradiotherapy versus radiotherapy alone in women with high-risk endometrial cancer (PORTEC-3): patterns of recurrence and post-hoc survival analysis of a randomised phase 3 trial. Lancet Oncol. 2019;20(9):1273-85.
18. Randall M, Filiaci V, McMeekin D, von Gruenigen V, Huang H, Yashar C, et al. Phase III Trial: Adjuvant Pelvic Radiation Therapy Versus Vaginal Brachytherapy Plus Paclitaxel/Carboplatin in High-Intermediate and High-Risk Early Stage Endometrial Cancer. J Clin Oncol. 2019;37(21):1810-8.
19. Randall M, Filiaci V, Muss H, Spirtos N, Mannel R, Fowler J, et al. Randomized phase III trial of whole-abdominal irradiation versus doxorubicin and cisplatin chemotherapy in advanced endometrial carcinoma: a Gynecologic Oncology Group Study. J Clin Oncol. 2006;24(1):36-44.
20. Matei D, Filiaci V, Randall M, Mutch D, Steinhoff M, DiSilvestro P, et al. Adjuvant chemotherapy plus radiation for locally advanced endometrial cancer. N Engl J Med. 2019;380(24):2317-26..

Corresponding author:

Georgios Androutsopoulos MD
Assistant Professor, Department of Obstetrics and Gynaecology, University of Patras, Medical School, Rion 26504, Greece
Tel: +30 6974 088092
E-mail: androutsopoulos@upatras.gr, androutsopoulosgeorgios@hotmail.com

ORIGINAL RESEARCH ARTICLE ACHAIKI IATRIKI | 2021; 40(1):28–33

An in-house procedure for the isolation of total RNA for SARS-CoV-2 detection in nasopharyngeal or oropharyngeal specimens by quantitative real time PCR

Maria Rodi, Tassos Georgakopoulos, Fotini Kalogianni, Athena Alexandropoulou, Anne-Lise de Lastic, Ioannis Panagoulias, Athanasia Mouzaki

Abstract

SARS-CoV-2, the virus responsible for the ongoing COVID-19 outbreak, is a positive single-stranded RNA virus similar to existing SARS-CoV viruses, although evolutionarily distant. To date, diagnosis for COVID-19 has been based on the detection of SARS-CoV-2 in nasopharyngeal or oropharyngeal specimens using quantitative real-time PCR (qRT-PCR). The mortality rates and rapid spread of SARS-CoV-2 have led to a high demand for commercially available kits and reagents for the extraction of viral RNA and subsequent qRT-PCR analysis. From the experience of many laboratories, including our own, there is often a shortage of kits, particularly those for viral RNA extraction. We report here a protocol we developed for RNA extraction for the detection of SARS-CoV-2 by qRT-PCR. Our method uses reagents commonly found in molecular biology research laboratories that are readily available and inexpensive. Comparison of our in-house method with an automated total RNA extraction method using a widely available commercial kit showed that we consistently obtained the same or better results with our in-house RNA extraction protocol.

Key words: *COVID-19; RNA extraction; quantitative real time PCR*

INTRODUCTION

The novel coronavirus SARS-CoV-2, which emerged in Wuhan, China, since December 2019, has spread rapidly worldwide, infecting over 94.5 million people, of whom 2,022,279 have died [1].

SARS-CoV-2 is a positive single-stranded RNA virus that is similar to previous SARS-CoV viruses, although it is evolutionarily distant. The SARS-CoV-2 ssRNA encodes proteins with distinct roles, including the spike glycoprotein, which is responsible for cell adhesion and entry via the host cell receptor ACE2, and Nsp1, which acts as an antagonist of the IFN response and induces chemokine secretion [2-6].

In patients with COVID-19, lymphopenia, mainly due to activation-induced T-cell death, accompanied by reduced numbers of regulatory T-cells and significant upregulation of the cytokines IL-1β, IL-6, TNF-α, IL-10 and the chemokines MCP1, IP-10, MIP1A, MIP1B, is associated with poor outcome [7,8].

To date, diagnosis has been based mainly on the detection of SARS-CoV-2 in nasopharyngeal or oropharyn-

Laboratory of Molecular Diagnosis of Infectious Agents, Medical School, University of Patras, Patras, Greece

Received: 30 Nov 2020; Accepted: 18 Jan 2021

geal specimens by qRT-PCR [9]. The mortality rates and rapid spread of SARS-CoV-2 led to a high demand for commercially available kits and reagents for viral RNA extraction and qRT-PCR analysis. From the experience of many laboratories, including our own, there is often a shortage of kits and reagents, particularly those for viral RNA extraction. We report here a protocol that we have developed in our laboratory for SARS-CoV-2 RNA extraction to be used for SARS-CoV-2 detection by qRT-PCR. Our method uses reagents commonly found in molecular biology research laboratories that are readily available and inexpensive.

MATERIALS AND METHODS

Ethics

The Laboratory of Molecular Diagnosis of Infectious Agents, Medical School, University of Patras, Patras, Greece, is one of the COVID-19 reference laboratories in Greece that performs SARS-CoV-2 detection by qRT-PCR in nasopharyngeal and oropharyngeal samples collected by hospital staff or health care workers.

The Laboratory performs these tests for the 6th Health District of Greece (Southwest Greece and Ionian Sea islands). The Laboratory was established by Resolution no. 217/9186/12.3.2020 of the Extraordinary Session of the Senate of the University of Patras 164/12.3.2020, which was published in the Government Gazette on 21/03/2020, no. 955.

The methodology described in this study and the data analyzed are part of a service provided by the Laboratory to the Hellenic National Public Health Organization (EODY). EODY and the Greek government encourage the official COVID-19 laboratories to develop their own protocols for RNA extraction and SARS-CoV-2 detection by qRT-PCR, to increase the diagnostic adequacy of the country in case of shortages of commercially available kits and reagents for viral RNA extraction and qRT-PCR analysis.

Case demographics and test results are sent electronically to EODY in encrypted files that can be opened with a code known only to the laboratory director and designated EODY staff. This procedure has been approved by the Hellenic Data Protection Authority. For the purpose of the current study, informed consent was not required.

Samples

Nasopharyngeal and oropharyngeal samples are collected in tubes containing Dulbecco's Modified Eagle's Medium (DMEM) (Copan swabs #330C USA; Deltalab Swab Virus #304297 and #304291 Spain; MicroBiotech swabs 250/VIR/AL, Italy) and stored at 4oC until they reach our laboratory. Samples are either processed immediately or stored at -80oC until processing. Sample handling and RNA extraction takes place in a biosafety level III laboratory in a type II hood in a clean room. qRT-PCR analysis takes place in a separate laboratory. Leftover biological and RNA samples are stored at -80oC. For this work, 6 frozen biological samples were used, including 4 positive and 2 negative for SARS-CoV-2, as previously determined using commercial kits, as described below.

In-house method for total RNA extraction

An amount of 300 μl per biological sample was transferred to a 1.5 ml Eppendorf-type centrifuge tube containing 700 μl Trizol (T9424 Sigma, Germany), mixed x5 by inversion, and left to stand for 5 min at RT. Next, 200 μl chloroform was added, mixed x5 by inversion, and allowed to stand for 3 min at RT. The tubes were centrifuged at 12,000g at 4oC for 15 min. From the supernatants, 500-600 μl/sample were transferred to new tubes to which 600 μl of ice-cold isopropanol was added. The tubes were mixed x5 by inversion and allowed to stand at -80oC for 10 min. The tubes were then centrifuged at 12,000g at 4oC for 10 min. After removing the supernatant, RNA pellets were diluted in 75% ice-cold ethanol, and the tubes were centrifuged at 12,000g at 4oC for 5 min. The ethanol was removed and the RNA pellets were dried for 10 min at RT. Next, 12 μl of nanopure water was added to each tube, and the tubes were incubated at 37oC for 10-15 min. The RNA concentration and purity in the aqueous solution was determined using a micro-volume UV-Vis Spectrophotometer Q5000 (Quawell Technology, Inc. USA). The method in protocol format is shown in Table 1.

Automated total RNA extraction

Automated total RNA extraction was performed with the Q-QiaSymphony Nucleic Acid extractor (Qiagen, Germany) using the QIA.937036 QIAsymphony DSP Virus/Pathogen Mini Kit (Qiagen, Germany) according to the manufacturer's instructions [10, 11]. The final volume of each RNA sample was 50 μl. For the purpose of this study RNA concentration and purity were determined as described above.

cDNA preparation and quantitative RT-PCR

The preparation of cDNAs from both in-house and automated extracted RNAs and subsequent qRT-PCR was performed using the one-step reverse transcription and RT-PCR detection kit VS-NCO296 VIASURE SARS-CoV-2

Table 1. *Step-by-step method for total RNA extraction.*

Step	Procedure
1.	Prepare 1.5 ml Eppendorf-type centrifuge tubes containing 700 μl Trizol (we use T9424 from Sigma, Germany)
2.	Transfer 300 μl of each biological sample into the tubes
3.	Mix x5 by inverting
4.	Leave the tubes for 5 min at RT
5.	Centrifuge the tubes for 15 min at 12,000g at 4oC
6.	Transfer 500-600 μl of each supernatant to new tubes
7.	Add 600 μl of ice-cold isopropanol to each tube
8.	Mix x5 by inverting
9.	Transfer the tubes to -80oC and leave for 10 min
10.	Centrifuge tubes for 10 min at 12,000g at 4oC
11.	Remove supernatant
12.	Dilute RNA pellets in 75% ice-cold ethanol
13.	Centrifuge the tubes for 5 min at 12,000g at 4oC
14.	Remove the ethanol and allow the RNA pellets to dry for 10 min at RT
15.	Add 12 μl of nano-pure water to each tube
16.	Incubate the tubes for 10-15 min at 37oC
17.	Determine the RNA concentration and purity in the aqueous solution (we use a microvolume UV-Vis Spectrophotometer Q5000 (Quawell Technology, Inc. USA)

(CerTest Biotec, S.L., Spain) [12]. For the reaction, 5 μl of each RNA sample was added to 15 μl of the rehydrated reaction mix of the kit containing all the necessary factors for reverse transcription and PCR. Reverse transcription and qRT-PCR were performed on a Rotor-Gene Q MDx 5plex Platform RT-PCR cycler (Qiagen, Germany). The program for the reactions was: 15 min at 45ºC for reverse transcription, 2 min at 95ºC for initial denaturation of cDNAs and then for 45 cycles the following two steps, 10 sec at 95oC for denaturation and 50 sec at 60oC for annealing/extension.

RESULTS AND DISCUSSION

We compared the qRT-PCR results of 6 total RNAs extracted from biological samples using either QIAsymphony or our own method. Total RNAs extracted with QIAsymphony were in a total volume of 50 μl/sample. For the in-house method, total RNA was first diluted in a small volume (12 μl/sample), and serial dilutions were performed. The concentration of all RNA samples was determined spectrophotometrically (Table 2).

As shown in Table 2, the initial concentrations (A) of all RNAs prepared by the in-house method were higher than those prepared by QIAsymphony. The initial concentrations (A) of RNAs prepared by the in-house method were between 10-fold (P1) and 13-fold (P3) higher than those prepared by QIAsymphony. The final dilution concentrations (D) of the RNAs prepared in-house were closer to the concentrations of the RNAs prepared with QIAsymphony. Using the in-house method, extraction of total RNA takes approximately 1 h and 15 min for 6 biological samples.

For each qRT-PCR reaction, 5μl per RNA sample (diluted or undiluted) was used. Detection of SARS-CoV-2 using Viasure Real Time PCR Detection Kit is based on amplification of a conserved region of ORF1ab

Table 2. *Concentrations of total RNAs prepared by the in-house method vs QIAsymphony.*

Sample	Type of RNA extraction				
	QIAsymphony	In-house method			
		A	B=A/2	C=B/2	D=C/2
P1	19.76	110.24	55.12	27.56	13.78
P2	22.92	328.16	164.08	82.04	41.02
P3	52.88	671.08	335.54	167.77	83.885
P4	25.56	448.2	224.1	112.05	56.025
N1	27.32	499.32	249.66	124.83	62.415
N2	25.68	417.76	208.88	104.44	52.22

RNA concentrations are in ng/μl; P, positive sample, N, negative sample. A, initial concentration of RNAs prepared by the in-house method. B, C and D are serial dilutions of A.

and N genes of SARS-CoV-2 with specific primers and fluorescent-labeled probes. In the Rotor-Gene Q MDx RT-PCR cycler, the N gene is amplified and detected in the ROX channel, the ORF1ab gene in the FAM channel, and the internal control (IC) in the HEX channel.

As shown in Table 3, sample P3 did not yield results for the virus N and ORF1ab genes or the internal control at the initial concentration (A); this sample yielded positive qRT-PCR results at lower concentrations. In reverse transcription reactions it is common to have a decrease of efficiency due to an excess of the starting template. High concentration templates probably contain greater amounts of reverse transcription and/or PCR inhibitors that limit subsequent steps.

Four RNA samples yielded positive results and 2 negative, at all concentrations used. Regardless of the RNA preparation method used or its concentration, the cycle thresholds (ct) were nearly identical for all targets tested (N gene, ORF ab gene and IC) (Table 3). In Figure 1, we show characteristic results of qRT-PCR of 2 positive samples (P1, P3) and 1 negative sample (N).

We repeated the in-house RNA extraction procedure for an additional 200 randomly selected positive and negative samples that we had in stock. For the additional samples, we decided to use a range of RNA concentrations between 1.2-264.2 ng/ml of sample (median concentration, 116.59 ng/ml) and we obtained the same results obtained with the QIAsymphony

Table 3. *Ct values for SARS-CoV-2 gene targets and internal control.*

Sample	N gene Ct				
	Type of RNA extraction				
	QIAsymphony	In-house method			
		A	B	C	D
P1	29.44	28.21	29.46	30.22	31.03
P2	25.9	27.78	28.7	29.16	30.71
P3	19.53	-	18.07	19.34	20.02
P4	19.51	20.38	20.49	21.4	22.61
N1	-	-	-	-	-
N2	-	-	-	-	-
	ORF1 ab gene Ct				
P1	26.85	25.55	27.4	28.42	29.07
P2	22.23	30.2	26.5	27.78	29.17
P3	16.34	-	15.65	16.6	17.41
P4	15.83	20.34	18.7	19.45	20.65
N1	-	-	-	-	-
N2	-	-	-	-	-
	IC Ct				
P1	20.69	20.16	20.49	20.59	20.4
P2	18.9	17.59	18.3	18.51	18.32
P3	18.62	22.32	17.32	18.12	18.13
P4	17.02	19.14	19.79	20.26	20.16
N1	22.1	18.03	18.63	19.19	19.19
N2	22.38	17.99	19.07	19.38	19.3

RNA concentrations are in ng/μl; Ct, Cycle threshold; IC, Internal Control; P, positive sample; N, negative sample; A denotes the initial concentration of total RNAs prepared by the in-house method; B, C and D are serial dilutions of A.

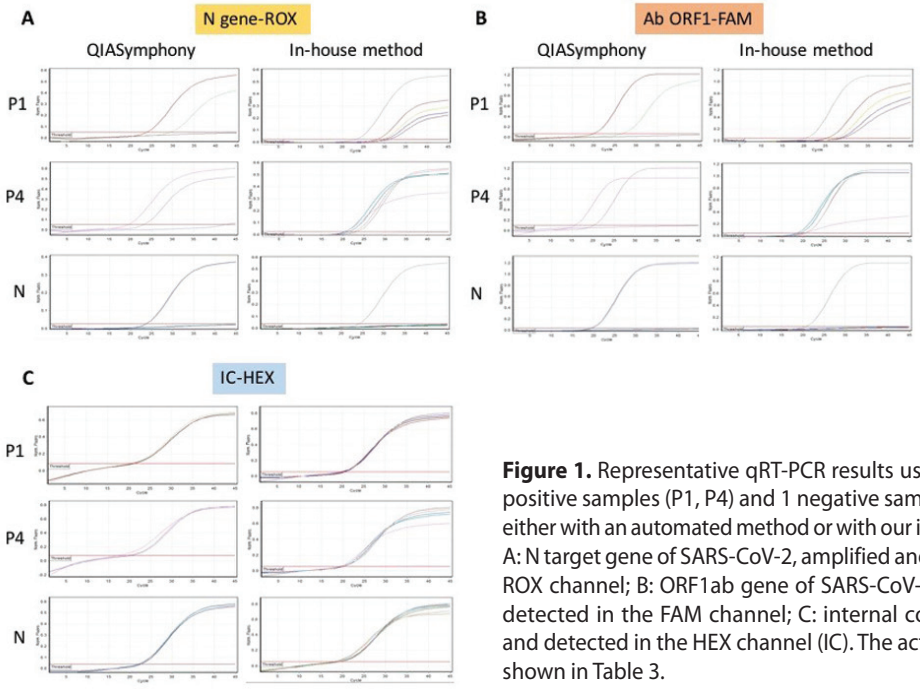


Figure 1. Representative qRT-PCR results using RNAs from 2 positive samples (P1, P4) and 1 negative sample (N) prepared either with an automated method or with our in-house method. A: N target gene of SARS-CoV-2, amplified and detected in the ROX channel; B: ORF1ab gene of SARS-CoV-2, amplified and detected in the FAM channel; C: internal control, amplified and detected in the HEX channel (IC). The actual ct values are shown in Table 3.

method. In addition, we were able to extract RNA from biological samples that could not be used for the QIAsymphony method due to too high viscosity or too low volume.

CONCLUSIONS

In conclusion, our in-house method for RNA extraction is standardized to perform as well as or better than a widely used automated method for RNA extraction. Our method uses reagents commonly found in molecular biology research laboratories that are readily available and cheap to buy, and it yields higher amounts of total RNA, which can be very helpful when the quality of biological samples is poor and/or their quantity is very low.

So far, massive screening of the population for SARS-CoV-2 by qRT-PCR combined with a policy of social distancing is the only protection against the infection. As vaccination against SARS-CoV-2 [13, 14] begins, molecular screening will continue to determine whether herd immunity develops and whether vaccinated individuals are immune to new SARS-CoV-2 infections.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *This work was funded by the University of Patras Special Account for Research Funds Program Coronavirus COVID-19 #81198.*

Author contributions: *AM conceived and coordinated the study, MR, FK, AA, ALdL performed the experiments, TG advised on methodology, MR and TG analyzed the data and prepared the tables and figure, MR, TG, IP and AM wrote the manuscript. AM revised the manuscript. All authors approved the revised manuscript.*

REFERENCES

1. Johns Hopkins Coronavirus Resource Center. "COVID-19 Map." Accessed January 17, 2021. Available from: https://coronavirus.jhu.edu/map.html.
2. Chan JF, Kok KH, Zhu Z, Chu H, To KW, Yuan S, et al. Genomic characterization of the 2019 novel human pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg Microbes Infect. 2020;9(1):221-236.
3. Andersen KG, Rambaut A, Lipkin WI, Holmes, EC, Garry RF. The proximal origin of SARS-CoV-2. Nat Med. 2020;26(4):450-452.
4. Anastasopoulou S, Mouzaki A. The biology of SARS-CoV-2 and the ensuing COVID-19. Achaiki Iatriki. 2020;39(1):29-35.
5. Stancioiu F, Papadakis G, Kteniadakis S, Nikovaevich Izotov B, Coleman M, Spandidos D, et al. A dissection of SARS-CoV2 with clinical implications. Int J Mol Med. 2020;46(2):489-508.
6. Docea A, Tsatsakis A, Albulescu D, Cristea O, Zlatian O, Vinceti M, et al. A new threat from an old enemy: Re-emergence of coronavirus. Int J Mol Med. 2020;45(6):1631-1643.
7. Qin C, Zhou L, Hu Z, Zhang S, Yang S, Tao Y, et al. Dysregulation of Immune Response in Patients With Coronavirus 2019

(COVID-19) in Wuhan, China. Clin Infect Dis. 2020;71(15):762-768.
8. Del Valle DM, Kim-Schulze S, Huang H-H, Beckmann ND, Nirenberg S, Wang B, et al. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat. Med. 2020;26(10):1636-1643.
9. Corman VM, Landt O, Kaiser M, Molenkamp R, Meijer A, Chu DKW, et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):2000045.
10. QIAsymphony SP Protocol Sheet- Complex200_OBL_V4_DSP protocol 12/2017 www.giagen.com
11. QIAsymphony DSP Virus/Pathogen Mini Kit handbook 06/2015 www.giagen.com
12. VS-NCO296 VIASURE SARS-CoV-2 RT-PCR Detection Kit handbook www.certest.es
13. Koirala A, Joo YJ, Khatami A, Chiu C, Britton PN. Vaccines for COVID-19: The current state of play. Paediatr Respir Rev. 2020;35:43-49.
14. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, et al. Safety and Efficacy of the BNT162b2 mRNA COVID-19 Vaccine. N Engl J Med. 2020;383(27):2603-2615.

Corresponding author:

Athanasia Mouzaki
Laboratory of Molecular Diagnosis
of Infectious Agents, Medical School
University of Patras, Patras, Greece
Tel.: +30 2610 969123, E-mail: mouzaki@upatras.gr
ORCID: 0000-0001-5548-7002

ORIGINAL RESEARCH ARTICLE ACHAIKI IATRIKI | 2021; 40(1):34–37

The use of non-invasive ventilation in respiratory patients in a tertiary general hospital

Marianna Maliouki[1]*, Dimosthenis Lykouras[1]*, Kiriakos Karkoulias[1], Heleni Loutrari[2], Stylianos Orfanos[3], Kostas Spiropoulos[1], Anastasia Kotanidou[2]

Abstract

Background: Non-Invasive Ventilation (NIV) is increasingly becoming more popular as a useful treatment modality of acute respiratory failure prior to intubation in critically ill patients. The aim of this study was to investigate the effect and outcome of treatment of patients suffering from lung disease, who were provided non-invasive ventilation (NIV) in the respiratory department of a tertiary general hospital.

Methods: In this retrospective study, a combined analysis of demographic characteristics, age, duration of hospitalization, duration of use of NIV, outcome of the disease, symptoms and treatment method was performed. Data were processed to detect factors that affect the outcome of the disease with the use of NIV.

Results: Data from 95 patients who had been hospitalized during a 2-year period in the respiratory department of a tertiary hospital were examined. Data analysis showed that the two most common diseases that led to the use of NIV was pneumonia and other respiratory infections, with no significant variation among different age groups. Comorbidities played an important role on hospitalization length and outcome.

Conclusion: In this study, the most common indication for the use of NIV was respiratory failure due to respiratory infection. Older patients with multiple comorbidities required longer hospitalization and had a worse outcome.

Key words: *NIV; CPAP; respiratory failure; pneumonia; COPD*

INTRODUCTION

Since 1960, the use of invasive mechanical ventilation following intubation has been used for the treatment of critically ill patients, especially to protect the airways and provide ventilatory support. However, in the latest decades non-invasive mechanical ventilation (NIV) is increasingly used in severely ill patients as a measure to support the respiratory system [1,2]. It has been proven effective in reducing in-hospital morbidity and mortality, reducing hospitalization time and cost. NIV is also facilitating the weaning procedure in Intensive Care Units (ICU) [3,4,5].

Non-invasive ventilation is the use of mechanical ventilation that does not require intubation [6]. It was initially used in chronic respiratory failure in respiratory patients, but it is now also used in acute respiratory failure settings (Acute respiratory failure, ARF) [7]. NIV has been used for the treatment of acute lung injury (ALI), acute respiratory distress syndrome (ARDS), acute exacerbation of chronic obstructive pulmonary disease (AE-COPD), obesity hypoventilation syndrome (OHS) and

[1]Department of Respiratory Medicine, University Hospital of Patras, Rio Patras, Greece
[2]First Department of Critical Care Medicine & Pulmonary Services, Evangelismos Hospital, National and Kapodistrian University of Athens Medical School, Athens, Greece
[3]Second Department of Critical Care, Attikon Hospital, National and Kapodistrian University of Athens Medical School, Athens, Greece
*Equal authors

Received: 24 Sep 2020; Accepted: 17 Dec 2020

pulmonary oedema in patients suffering from congestive heart failure [8,9].

The main reason that NIV has been popular amongst clinicians is the fact that it can be used even outside the ICU in critically ill patients, helping to reduce the risk of intubation in certain patient groups [10]. Therefore, several major complications of invasive mechanical ventilation can be avoided. By using NIV, the airways remain intact, the normal preventive mechanisms remain active, the patient can speak, eat, and drink normally. Moreover, ventilator-associated infections can be less frequent, and the patient remains awake and co-operative. A study comparing NIV to invasive mechanical ventilation, has shown that NIV is associated with lower risk of in-hospital infection, reduced use of antibiotics, reduced ICU admission and lower mortality [11].

The aim of this study was to evaluate the use of Non-Invasive Ventilation (NIV) in critically ill patients in the Pneumology Department of a tertiary general hospital in Greece.

MATERIALS AND METHODS

This was a retrospective study that used data of 2 consecutive years (Jan 2015 - Dec 2016) from hospitalized patients in the Pneumology Department of a tertiary general hospital in Greece. As a retrospective study by design, this study did not by any means interfere with patients' treatment selection by any means. All patient information was retracted from patient case files and was subsequently processed. All collected data were anonymized and were safely stored in a dedicated database until they were analyzed.

Statistical analysis

Clinical and demographic characteristics were compared using student's t-test. All analyses were performed using the IBM SPSS software for windows (SPSS 25.0). Statistical significance level was set as usual at p-values <0.05.

RESULTS

A total of 95 patients entered this study, 55 men (57%) and 40 women (42%). Mean age was 70 years, with a range between 28 and 96 years old. 18 patients had to be excluded from analysis due to incomplete data.

Most of the patients receiving NIV treatment belonged to the 61-80 years old age-group, followed by the 41-60 years old age-group and the 81-100 years old age-group. Only 1 patient was between 21-40 years old. The age distribution did not differ between men and women ($p = 0.348$) (Table1).

The mean hospitalization time was 24 days in these critically ill patients and the mean duration of NIV use was 9 days. The more commonly used NIV setting was continuous positive airway pressure (CPAP) in 72 patients (94%), while only 5 patients (6%) used bilevel positive airway pressure (Bi-PAP type or compatible).

The most common reason for hospitalization that required use of NIV was a respiratory infection (hypoxaemia and respiratory failure), which was present in 29 patients (31.5%). It was followed by pneumonia with chest x-ray infiltrates in 27 patients (29.3%) and AE-COPD in 19 patients (20.7%). In the pneumonia group, 20 out of the 27 cases were bacterial pneumonias with laboratory confirmation (Table 2). Finally, 6 patients fulfilled more than one indication for the use of NIV.

Hospitalization outcome was also investigated, and it was found that 65 patients had been discharged and

Table 1. *Patient characteristics and age distribution.*

Age	Male	Female	Total
21-40	1	0	**1**
41-60	9	12	**21**
61-80	21	18	**39**
91-100	11	5	**16**
Total	**42**	**35**	**77**

Table 2. *Indications for NIV use and percentages.*

Indication	No	%	Indication	No	%
Respiratory infection	29	31.5	ARDS	2	2.5
Pneumonia	27	29.3	Interstitial lung disease	1	1.2
AE-COPD	19	20.7	Rehabilitation after ICU	1	1.2
Asthma	4	5.0	Sleep apnoea	1	1.2
Acute respiratory failure	3	3.7			

12 patients had died.

The NIV indications across age groups were the following: pneumonia in the 21-40 years old age-group, AE-COPD in the 41-60 years old age-group and respiratory infection in the 61-80 years old and 81-100 years old age-groups.

Comorbidities in critically ill patients are always an issue and this was also shown in this study, with 56 patients from the study population having at least one or more comorbidities. The most common comorbidities were chronic respiratory failure, COPD, diabetes mellitus and coronary artery disease. Patients with >2 comorbidities needed more days in hospital than other patients ($p = 0,048$) (Tables 3, 4).

DISCUSSION

The use of NIV is increasingly considered as an initial alternative to mechanical ventilation in critically ill patients with acute respiratory failure with certain indications. The use of NIV in a CPAP setting and proper interface is considered a safe, easy to use and effective method of ventilation that can be used outside the ICU, in emergency department rooms and even in a hospital ward by qualified personnel.

Some of the prerequisites for its use are: 1) right indication, 2) proper interface mask, 3) co-operation of the patient and caregivers, 4) close monitoring, 5) experience of the medical and nurse team, 6) ICU back-up in case intubation is needed. Of course, in patients that fulfil criteria for early failure of NIV, all NIV procedures should be done in the ICU setting and not in a regular hospital ward.

Table 3. *Comorbidities and length of hospitalization and NIV use.*

Comorbidities	Mean length of hospitalization (days)	Mean length of NIV use (days)
1 comorbidity	20	8
2 comorbidities	17	10
>2 comorbidities	27	8

Table 4. *Comorbidities and outcome.*

Comorbidities	Discharge (n)	Death (n)
1 comorbidity	21	2
2 comorbidities	24	4
>2 comorbidities	20	6

Non-invasive ventilation use is well established in the management of respiratory failure secondary to an acute exacerbation of COPD. Randomized clinical trials have shown that NIV reduces the need for endotracheal intubation and may also improve survival [1]. The use of NIV in different clinical settings has been tested in clinical trials, with pneumonia and cardiogenic pulmonary oedema being common indications together with COPD exacerbations [2]. However, in cases of severe pneumonia (multiple lobes involved, hypotension, oxygenation index < 250 mmHg) NIV shows a high rate of failure. These patients should be carefully monitored with chest x-rays and arterial blood gas sampling, so that mechanical ventilation can be started if necessary [3].

In this study the most common indications for the use of NIV were respiratory infections. The impact of multiple comorbidities on hospitalization time and final outcome was also investigated, and a positive correlation was revealed between the presence of more than 2 comorbidities and the number of days in hospital and even in-hospital mortality rates. Older patients with more comorbidities were those that required more days under treatment and demonstrated a poorer outcome.

Finally, this study shows that the use of NIV in a Pneumology Department in a tertiary general hospital in Greece was according to recommendations of the European Respiratory Society (ERS) and the British Thoracic Society (BTS) and that the outcomes were as expected according to the findings of wide-scale trials around the world.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *None to declare.*

Author contributions: *MM: manuscript concept, data collection, data analysis, manuscript drafting, DL: manuscript concept, data collection, data analysis, manuscript drafting, KK: manuscript concept, manuscript drafting, HL: manuscript concept, manuscript drafting, SO: manuscript concept, manuscript revision, KS: manuscript concept, manuscript revision, AK: manuscript concept, manuscript revision*

REFERENCES

1. Plant PK, Owen JL, Elliott MW. Early use of non-invasive ventilation for acute exacerbations of chronic obstructive pulmonary disease on general respiratory wards: a multicentre randomised controlled trial. Lancet. 2000;355(9219):1931-5.

2. Köhnlein T, Windisch W, Köhler D, Drabik A, Geiseler J, Hartl S, et al. Non-invasive positive pressure ventilation for the treatment of severe stable chronic obstructive pulmonary disease: a prospective, multicentre, randomised, controlled clinical trial. Lancet Respir Med. 2014;2(9):698-705.
3. Burns KE, Meade MO, Premji A, Adhikari NK. Noninvasive positive-pressure ventilation as a weaning strategy for intubated adults with respiratory failure. Cochrane Database Syst Rev. 2013;(12):CD004127
4. British Thoracic Society Standards of Care Committee. Non-invasive ventilation in acute respiratory failure. Thorax. 2002;57(3):192-211.
5. Elliott M, Nava S, Schönhofer B. Non-Invasive Ventilation and Weaning: Principles and Practice, Second Edition 2nd Edition. CRC Press; 2010.
6. Elliott MW, Nava S. Noninvasive ventilation for acute exacerbations of chronic obstructive pulmonary disease: "Don't think twice, it's alright!". Am J Respir Crit Care Med. 2012;185(2):121-3.
7. Carrillo A, Ferrer M, Gonzalez-Diaz G, Lopez-Martinez A, Llamas N, Alcazar M, et al. Noninvasive ventilation in acute hypercapnic respiratory failure caused by obesity hypoventilation syndrome and chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2012;186(12):1279-85.
8. Nava S, Fanfulla F. Non Invasive Artificial Ventilation, Springer –Verlag, Italia; 2014.
9. Duarte AG, Justino E, Bigler T, Grady J. Outcomes of morbidly obese patients requiring mechanical ventilation for acute respiratory failure. Crit Care Med. 2007;35(3):732-7.
10. Elliott MW, Nava S. Noninvasive ventilation for acute exacerbations of chronic obstructive pulmonary disease: "Don't think twice, it's alright!". Am J Respir Crit Care Med. 2012;185(2):121-3.
11. Appendini L, Patessio A, Zanaboni S, Carone M, Gukov B, Donner CF, et al. Physiologic effects of positive end-expiratory pressure and mask pressure support during exacerbations of chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 1994;149(5):1069-76.
12. Díaz GG, Alcaraz AC, Talavera JC, et al. Noninvasive positive-pressure ventilation to treat hypercapnic coma secondary to respiratory failure. Chest. 2005;127(3):952-60.
13. British Thoracic Society Standards of Care Committee. Non-invasive ventilation in acute respiratory failure. Thorax. 2002;57(3):192-211.
14. Jolliet P, Abajo B, Pasquina P, et al. Non-invasive pressure support ventilation in severe community-acquired pneumonia. Intensive Care Med. 2001;27(5):812-21.

Corresponding author:

Kiriakos Karkoulias
Assistant Professor, Dept. of Respiratory Medicine,
University Hospital of Patras, Rio Patras, GR 26500, Greece
Tel.: +30 2610999523, Fax: +30 2610999523
E-mail: karkoulias@upatras.gr

ORIGINAL RESEARCH ARTICLE ACHAIKI IATRIKI | 2021; 40(1):38–43

Health resources allocation inequalities in a deprived Greek region: Differences between primary care and hospital sector

Christos Bartsokas, Eleni Jelastopulu

Abstract

Background: Accessibility in health care defines a universal health system. Our goal was to investigate the equality on the distribution of health resources, in the middle of economic crisis, between hospitals and primary care sector in the Western Greece region.

Methods: All data were provided by the Hellenic Statistical Authority for the years 2010 to 2013. Gini coefficients were calculated in order to measure inequality, ranging from 0 to 1, where 1 stand for the absolute inequality. Calculations were based on population size and geographic size, respectively, for the indicators: number of institutions, health workers and hospital beds.

Results: Distribution of health resources for hospital and primary care sector were reported for each prefecture in the Western Greece region separately and as a whole. The urban prefecture of Achaia demonstrated higher rates of resources in hospital sector, inversely more rural ones have had higher rates in primary health care resources. Gini coefficient ranged between 0.40 to 0.49 for hospitals, while in the primary care sector it was around 0.21, indicating a good equality in the distribution of resources.

Conclusion: Amidst economic crisis for Greece, inequality in the geographic distribution of health resources was evident, despite a more equitable per population distribution of resources. In the urban prefecture of Achaia, it is more likely to have access to well-resourced hospitals for outpatient care, while the lower rates of health resources in the primary care sector depict chronic systemic disparities. A focus on resources needed for specific health services will contribute to dealing with inequalities in order to achieve a universal health system.

Key words: *Inequality; health care; primary care sector; hospital sector*

INTRODUCTION

Universal health systems aim to provide health care for all, or to rephrase it, they aim on equal distribution of health resources based on peoples' needs [1,2]. Healthcare resources are defined as all materials, personnel, facilities, funds, and anything else that can be used for providing health care services. Equity in resource distribution requires that individuals with the same need have access to the same resources (horizontal equity) and that individuals with greater need have access to more resources (vertical equity). In 2010, WHO reported as one of the main barriers to universal health coverage the availability and inequitable use of resources [3].

In Greece, healthcare services are delivered by primary care institutions and hospitals. Primary care institutions which mainly focus on preventive health practices, seem to lack resources compared with hospitals treating medical conditions. Thus, more resources have been poured into hospitals, further exacerbating disparities between hospitals and primary care institutions [4,5]. In a generalized framework, the economic crisis which started in 2008, deepened inequalities in almost all as-

Department of Public Health, School of Medicine, University of Patras, University Campus, Rio, Greece

Received: 01 Oct 2020; Accepted: 22 Dec 2020

pects of social life [6] and had a negative influence on population health, especially mental health [7,8]. This is partly due to the psychological insecurity, stress and access to material goods, and partly as a consequence of lack of access to healthcare.

In the past, several studies have shown inequity in healthcare resources regarding specific health conditions [9]; or in a specific health care delivery system [10]. In our study, we investigated the differences in the distribution of healthcare resources between the primary health sector and hospitals in a Greek region in regard with geographic location and population size. This study's results can inform policy makers on how these resources are distributed among Western Greece, a region with one of the lowest gross domestic product in Greece [11]. Furthermore, since no previous study has dealt with the issue in Greece, the findings of the present study provide appropriate evidence for the future planning and management of health sector resources in order to improve access to health services in the country.

METHODS AND MATERIALS

Data were extracted by the Hellenic Statistical Authority for the time period between 2010 to 2013 [12]. Population and geographic size were reported for Western Greece as a whole and by prefecture. A detailed statistical yearbook included exclusively public hospitals and primary health centres along with information about the number of institutions and number of available beds and health workers per institution, reflecting health resources.

We used Gini coefficient as the indicator for measuring inequality in the distribution of health resources against population size and geographic area. This index calculation is based on Lorenz curve (figure 1), which is a graphical representation of the cumulative proportion of health resources against the cumulative proportion of geographic area or population size. Pivotal properties of Lorenz curve are, that it always starts at (0,0) and ends at (1,1) and cannot rise above the line of perfect equality of 450. It defines two areas in the level, area A, the area between the line of perfect equality and the observed Lorenz curve and area B, the one between the Lorenz curve and the line of perfect inequality. Thus, the Gini coefficient is the ratio of the area between the line of perfect equality and the observed Lorenz curve to the area between the line of perfect equality and the line of perfect inequality. We have used this formula for calculating Gini coefficient:

Gini coefficient = area A/(area A + area B)

Therefore, it could take values from 0 to 1. The higher the coefficient, the more unequal the distribution is. Thus, Gini coefficient values categorised as absolute equality (GI <0.2), high equality (GI=0.2-0.3), inequality (GI=0.3-0.4), high inequality (GI=0.4-0.6) and absolute inequality (GI >0.6) [13]. All data were analysed using SPSS v.25.0

RESULTS

Tables 1 and 2 show the distribution of health resources for hospitals and the primary care sector, respectively. The Achaia prefecture, the most developed one in the region, had a much higher density in the distribution of hospitals, beds and health workers in the hospital sector, while a more equitable distribution compared with other prefectures in the distribution of heath resources in the primary care sector. In addition, the Ilia and Etoloakarnania prefectures, reported a much lower distribution of resources in the hospital sector while in the primary care sector we had a relatively inversed picture with increasing rates of resources in both rural prefectures compared with the Achaia prefecture, which could be categorized as more industrialised.

Table 3 depicts that the Gini coefficients against population size ranged between 0.04 and 0.21 in the hospital sector: 0.21 for the number of hospitals, around 0.14 for the number of beds and 0.04 for the number of health workers, respectively, indicating a good equality. The primary care sector showed a slightly higher level of Gini coefficients. The distribution in the number of primary health care institutions, beds and health workers was equitable, with Gini coefficients ranging from 0.21 to 0.34.

No trend was found in Gini coefficients over the years from 2010 to 2013. However, relative inequality

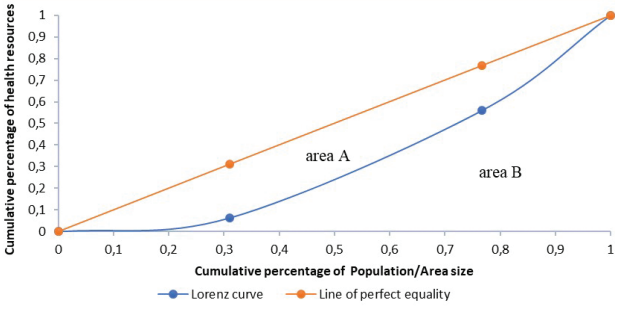


Figure 1. The straight line (orange line) depicts the line of perfect equality where resources are distributed evenly, while the curved line (blue line) shows the actual distribution of resources, giving a graphic representation of existed distribution inequality

Table 1. *Distribution of health resources for the years 2010 to 2013 in the hospital sector.*

Year		Prefecture		Hospitals		Beds		Health Workers	
		Population[1]	Area[2]	Population	Area	Population	Area	Population	Area
2010	Total	692.269	11.316	0.016	0.972	2.350	143.778	4.839	296.041
	Etoloakarnania	214.810	5.423	0.009	0.369	1.173	46.468	3.384	134.059
	Achaia	315.837	3.275	0.019	1.832	3.290	317.251	6.725	648.550
	Ilia	161.622	2.619	0.019	1.145	1.955	128.293	3.087	190.531
2011	Total	690.904	11.316	0.016	0.972	2.316	141.393	4.853	296.306
	Etoloakarnania	214.270	5.423	0.009	0.369	1.176	46.468	3.510	138.669
	Achaia	315.165	3.275	0.019	1.832	3.469	333.435	6.758	650.382
	Ilia	161.469	2.619	0.019	1.145	1.585	97.747	2.917	179.840
2012	Total	687.935	11.316	0.016	0.972	2.378	144.574	4.807	292.241
	Etoloakarnania	212.961	5.423	0.009	0.369	1.235	48.497	3.470	136.271
	Achaia	313.940	3.275	0.019	1.832	3.638	348.702	6.682	640.610
	Ilia	161.034	2.619	0.019	1.145	1.434	88.202	2.919	179.458
2013	Total	682.583	11.316	0.016	0.972	2.491	150.230	4.795	289.236
	Etoloakarnania	211.090	5.423	0.009	0.369	1.464	56.980	3.382	131.661
	Achaia	311.511	3.275	0.019	1.832	3.685	350.534	6.792	646.107
	Ilia	159.982	2.619	0.019	1.145	1.519	92.783	2.769	169.148

[1]Population: per 1000 persons; [2]Area: per 1000 km^2

was evident in the geographic distribution of health resources in hospitals. The Gini coefficients were between 0.40-0.49 in the geographic distributions of institutions, health workers and beds, indicating a higher level of inequality. The geographic distribution of primary care centres was equitable.

DISCUSSION

In our study, we investigated inequality in the distribution of health resources of hospital and primary sector in Western Greece, by using Gini coefficient. We found that inequality exists per geographic distribution of health resources in hospitals. We did not detect inequality either in the primary care sector or per population size. Health inequalities are generally understood to refer to differences in health between groups of people who are better or worse off socioeconomically, as reflected, for example, by their occupational status, income level, expenditures, wealth, or education, or by the economic characteristics of the places where they live. They are unfair and should be reduced by the right mix of government policies.

Not finding inequality in the primary care sector is not surprising, given that most resource planning programs have taken into consideration population size [14] and several reforms have been proposed since 1998 in the primary health care sector. Since Western Greece is a region with a medium geographic size but with the lowest Gross Domestic Product (GDP), defined as a standard measure of the value added created through the production of goods and services in an area during a certain period, in the country, this makes our results even more indicative of Greece's health related policies during the economic crisis [11,15].

Previous research in the field has demonstrated that a region with a high density of fairly distributed healthcare workforce is more likely to serve the healthcare needs of its people than a region with a low workforce density [16]. This can be seen in the primary sector in western Greece but not in hospitals per geographic size, probably due to the fact that despite their problems, the Greek primary healthcare centres represent the only organisational structure able to offer holistic services to clearly defined local populations

Table 2. *Distribution of health resources for the years 2010 to 2013 in the primary health care sector.*

Year		Prefecture		Primary care centres		Beds		Health Workers	
		Population[1]	Area[2]	Population	Area	Population	Area	Population	Area
2010	Total	692.269	11.316	0.029	1.767	0.149	9.102	0.381	23.329
	Etoloakarnania	214.810	5.423	0.047	1.844	0.214	8.482	0.507	20.100
	Achaia	315.837	3.275	0.016	1.527	0.101	9.770	0.288	27.786
	Ilia	161.622	2.619	0.031	1.909	0.155	9.546	0.396	24.437
2011	Total	690.904	11.316	0.030	1.856	0.149	9.102	0.359	21.916
	Etoloakarnania	214.270	5.423	0.047	1.844	0.219	8.667	0.452	17.887
	Achaia	315.165	3.275	0.016	1.527	0.086	8.244	0.260	25.038
	Ilia	161.469	2.619	0.037	2.291	0.180	11.072	0.427	26.346
2012	Total	687.935	11.316	0.031	1.856	0.129	7.865	0.332	20.237
	Etoloakarnania	212.961	5.423	0.047	1.844	0.197	7.745	0.470	18.440
	Achaia	313.940	3.275	0.016	1.527	0.073	7.023	0.197	18.931
	Ilia	161.034	2.619	0.037	2.291	0.149	9.164	0.416	25.582
2013	Total	682.583	11.316	0.031	1.856	0.136	8.218	0.243	14.669
	Etoloakarnania	211.090	5.423	0.047	1.844	0.180	7.007	0.370	14.383
	Achaia	311.511	3.275	0.016	1.527	0.074	7.023	0.170	16.183
	Ilia	159.982	2.619	0.038	2.291	0.200	12.218	0.219	13.364

[1]Population: per 1000 persons; [2]Area: per 1000 km^2.

[17]. While an equal distribution of beds and number of health care facilities (in both hospital and primary care centers) is documented in the literature, the number of healthcare professionals shows a much lower density in the primary care sector compared to the hospital sector, depicting thus the dominant role of the hospital sector in the Greek healthcare system [18-20].

In the present study, we focused on inequalities in health resources allocation and comparisons between the primary and the hospital sector. Existing literature is lacking information regarding those inequalities; we found only two studies from China and Ethiopia, pointing out inequalities between health system levels [18,21] and only the latter exclusively in the public health sector as it is stated in the present study. Similarly to our results, a much more developed hospital sector was prominent in China. Although some progress had been made with increased governmental investments in primary care, the capacity development of primary care still lags behind the hospital sector. Several other studies in China also found that quality resources tend to be increasingly concentrated in hospitals. In addition, as shown in our study, internal disparities within each economic zone further illustrate the regional differences in the equality of healthcare. The urban prefecture of Achaia has a much higher level of inequality compared with the other regions. Other studies tried to simplify the complexity in the nature of healthcare expenses distribution and revealed that high income countries demonstrated a lower intra-regional inequality compared with lower income ones [21,22], recognizing healthcare expenses as pivotal in minimizing inequalities.

A reduction in public spending in Greece between the years 2009 to 2013 by 30% deepened the gap between preventive medicine policies and curing diseases [23]. Thus, policies to support primary health care and decongest hospitals will strengthen the national health system instead of creating the impression of a two-tier system that opposes each other. More specifically, more health resources, especially quality health workers, should be allocated to primary care institutions in order to narrow the capacity gap between hospitals and primary care institutions. Secondly, regional disparities need to be addressed. This can only be done through

Table 3. *Gini coefficient for health resources distribution of hospitals and primary care centres against population and geographic area.*

Gini Coefficient	Year	Hospital Sector			Primary Care Sector		
		Institutions	Beds	Health Workers	Institutions	Beds	Health Workers
Population size	2010	0.21	0.15	0.04	0.30	0.25	0.22
	2011	0.21	0.14	0.06	0.30	0.29	0.25
	2012	0.21	0.15	0.06	0.30	0.28	0.29
	2013	0.21	0.12	0.07	0.31	0.34	0.21
Geographic size	2010	0.40	0.46	0.44	0.17	0.19	0.21
	2011	0.40	0.48	0.44	0.21	0.22	0.25
	2012	0.40	0.49	0.44	0.21	0.20	0.23
	2013	0.40	0.47	0.45	0.21	0.29	0.16

financial transfer coordinated by the central government. The current governmental budgeting system and the social health insurance arrangements in Greece are highly centralized and tethered, preventing the central government from fulfilling this role [24].

This study has some limitations as it analysed only a short period of time, in the middle of economic crisis. It would be interesting to perform further analyses on the longer-term changes, not only in Western Greece but throughout the country, when data are made available. We have selected those health resources indicators based on the availability by Hellenic Statistical Authority.

CONCLUSIONS

The proper and fair distribution of health resources, hospitals, beds and manpower, has a crucial role in delivering healthcare services. Inequality was found for health resources in the hospital sector in regard with geographic distribution, despite a more equitable per capita distribution of resources. The distribution of the primary health sector resources in Western Greece demonstrated a good equality, too. In addition, an existing gap in resources rates between urban and rural prefectures, was revealed; depicting the fact that people living in an urban area are more likely to use well-resourced hospitals than people living in rural areas. Further research should identify inequalities in resources needed for the deliverance of specific health services. In this way, the adjustment of specific healthcare policies will lead to a fairly equal distribution of health resources as a milestone in the development of a universal healthcare system.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *None to declare.*

Author contributions: *Christos Bartsokas: conception and design; analysis and interpretation of the data; drafting of the article; critical revision of the article for important intellectual content; final approval of the article, Eleni Jelastopulu: conception and design; critical revision of the article for important intellectual content; final approval of the article.*

REFERENCES

1. Daniels N, Kennedy BP, Kawachi I. Why justice is good for our health: the social determinants of health inequalities. Daedalus 1999;128(4):215–251.
2. Feinberg J. Encyclopedia of Bioethics: Justice. New York: Macmillan; 1995.
3. World Health Organization. The world health report Health systems financing: the path to universal coverage. Geneva: The World Health Organization; 2010. Available from: https://www.who.int/whr/2010/en/
4. Lionis C. Primary care in Greece. European forum for primary care; 2011.
5. The National Organization for the Provision of Health Services (Greek acronym EOPYY). Available from: https://eu-healthcare.eopyy.gov.gr/en/2_1.aspx
6. Karanikolos M, Kentikelenis A. Health inequalities after austerity in Greece. International Journal for Equity in Health. Int J Equity Health. 2016;15:83.
7. Bouras G, Lykouras L. The economic crisis and its impact on mental health. Encephalos. 2011;48:54-61
8. Karamanoli E. Debt crisis strains Greece's ailing health system. Lancet. 2011;378(9788):303–304.
9. Yuan S, Hong Y, Klemetti R, Qiang L, Shengbin X, Jianmin G,

et al. Equity in use of maternal health services in western rural China: a survey from Shaanxi province. BMC Health Serv Res. 2014;14:155.
10. Ameryoun A, Meskarpour-Amiri M, Dezfuli-Nejad ML, Khoddami-Vishteh H, Tofighi S. The assessment of inequality on geographical distribution of non-cardiac intensive care beds in Iran. Iran J Public Health. 2011;40(2):25-33.
11. Organisation for Economic Co-operation and Development. Regions and Cities in Greece; 2018. Available from: https://www.oecd.org/cfe/GREECE-Regions-and-Cities-2018.pdf
12. Hellenic Statistical Authority. Digital Library. Available from: http://dlib.statistics.gr/portal/page/portal/ESYE/
13. Miao CX, Zhuo L, Gu YM, Qin ZH. Study of large medical equipment allocation in Xuzhou. J Zhejiang Univ Sci B. 2007;8(12):881-4.
14. Lostao L, Blane D, Gimeno D, Netuveli G, Regidor E. Socioeconomic patterns in use of private and public health services in Spain and Britain: implications for equity in health care. Health Place. 2014;25:19-25
15. Administrative Regions of Greece. Available from: https://en.wikipedia.org/wiki/Administrative_regions_of_Greece
16. Anand S. Measuring health workforce inequalities: methods and application to China and India. World Health Organization; 2010. Available from: https://apps.who.int/iris/handle/10665/44417
17. Benos A. Primary health care by 2000. Iatrika Themata. 1999;16:12–15.
18. Zhang T, Xu Y, Ren J, Sun L, Liu C. Inequality in the distribution of health resources and health services in China: hospitals versus primary care institutions. Int J Equity Health. 2017;16(1):42.
19. Stordeur S, Leonard C. Challenges in physician supply planning: the case of Belgium. Hum Resour Health. 2010;8:28.
20. Boutsioli Z. The Greek Hospital Sector and Its Cost Efficiency Problems in Relation to Unexpected Hospital Demand: A Policy-making Perspective. Rev Eur Stud 2010;2:170.
21. Woldemichael A, Takian A, Sari A, Olyaeemanesh A. Inequalities in healthcare resources and outcomes threatening sustainable health development in Ethiopia: panel data analysis. BMJ Open 2019;9(1):e022923
22. Lessmann C. Regional inequality and decentralization: an empirical analysis. Environment and Planning A 2012;44(6):1363–88.
23. Organisation for Economic Co-operation and Development. Health Policy in Greece; 2016. Available from: http://www.oecd.org/greece/Health-Policy-in-Greece-January-2016.pdf
24. Skalli A, Johansson E, Theodossiou I. "Are the Healthier Wealthier or the Wealthier Healthier? The European Experience", Helsinki: ETLA. The case of Greece; 2006

Corresponding author:

Christos Bartsokas
Department of Public Health, School of Medicine,
University of Patras, 26500 Rio, Greece
Tel.: +30 2610 969878, Fax: +30 2610 996101
E-mail: bartsokas@gmail.com

ORIGINAL RESEARCH ARTICLE ACHAIKI IATRIKI | 2021; 40(1):44–48

Pyelonephritis, complicated by abscess formation, in young women, in the context of daily clinical praxis. Which is the best work-up imaging protocol? A mini pictorial review

Aikaterini Solomou[1], Pantelis Kraniotis[1], Nikolaos Ntoulias[2], Anastasios Apostolos[2], Irene Tsota[3], Aspasia Rigopoulou[1]

Abstract

Background: Renal abscess is a rare complication of acute pyelonephritis. Radiologic imaging is important, bearing in mind the lack of laboratory tests' specificity for the diagnosis of an abscess. It mostly occurs in diabetes mellitus patients (75% of abscesses) and/or in the existence of a stone, with young women being the most affected population. Our goal was to highlight the optimal imaging protocol, in the work-up of complicated pyelonephritis in young women, trying to avoid radiation exposure.

Methods: Four (4) cases of renal abscess following acute pyelonephritis in women of relatively young age, ranging from 17 to 22, who were admitted to our hospital in a 18-months period. All patients were submitted to ultrasound (U/S) examination and one patient to Magnetic Resonance Imaging (MRI).

Results & Conclusions: Because of its availability and low cost, U/S appears to be the first-line imaging technique for the investigation of a renal abscess after acute pyelonephritis. Particularly in cases of young women where imaging findings or laboratory tests are inconclusive, MRI with diffusion-weighted images (when available) should be used as an adjunct to confirm the abscess, obviating the need of radiation exposure.

Key words: *Renal abscess; ultrasonography; magnetic resonance imaging; diffusion weighted imaging; pyelonephritis; women's health*

INTRODUCTION

Pyelonephritis is one of the many faces of Urinary Tract Infection (UTI) and the formation of renal abscess is considered a rare clinical complication [1]. It mostly occurs in diabetes patients (75% of abscesses) and/or with coexisting renal calculi with young women being the most affected population [2]. Fortunately, the improvement of imaging techniques (Ultrasound (U/S), Computed Tomography (CT) and Magnetic Resonance Imaging (MRI) in combination with the advancement of

[1]MR Department, University General Hospital of Patras, Patras, Greece
[2]Department of Medicine, University of Patras, Patras, Greece
[3]Ultrasound Department, General Hospital of Patras, Patras, Greece

Received: 11 Oct 2020; Accepted: 27 Jan 2021

medical treatment and the wide application of interventional techniques, like percutaneous abscess drainage, have significantly decreased mortality rates from 40-50% to about 1.5%-15% in the last three decades [3].

We herein report a case series of renal abscesses in young women, highlighting the most prevalent imaging features and stressing-out the necessary imaging protocol, in order to avoid radiation exposure.

MATERIALS AND METHODS

We detected four cases of renal abscesses following acute pyelonephritis between May 2018 and November 2019 from our hospital archives. All patients were Caucasian women of young age, ranging from 17 to 24 years old, that were admitted to our hospital due to clinical manifestations resembling those of UTI and especially pyelonephritis. The right kidney was affected in 3 of the 4 cases. Patients did not present any other comorbidity, chronic medical treatment or had a history of a surgical procedure.

After a clinical examination and relevant laboratory tests, all patients were initially imaged with U/S scan [5]. In one case, with inconclusive clinical and lab tests, further imaging was needed. The patient was pregnant and could not be submitted to CT. In order to confirm the diagnosis, the latter patient was submitted to MRI, with DWI, without intravenous Gadolinium administration.

RESULTS

U/S

Patients were scanned with a convex probe and both grayscale and color Doppler images were acquired. The location of the abscesses was mainly cortical. Largest dimensions ranged between 1.5 to 2.4cm. The lesions were mainly hypoechoic. On color Doppler there was no vascularity within the lesions, apart from one where there was minimal internal vascularity. All cases presented with a small amount of perirenal fluid. Renal calculus was detected in one case. Ultrasound findings are summarized in Table 1. Ultrasound imaging findings are highlighted in Fig. 1-4.

MRI

The MRI protocol included T2WI TSE coronal and axial planes, T2WI TSE axial with fat- suppression images; Diffusion balances 0, 50, 100, 150, 500, T1 GRE with fat suppression axial and coronal images and T1WI GRE

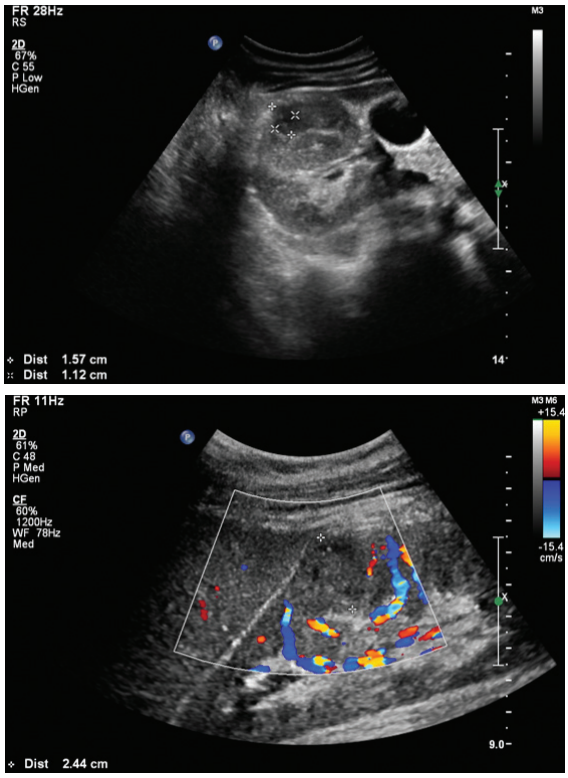


Figure 1 (a-b). Patient #1. Ultrasound images, grayscale (a) and color doppler (b) show a well-defined inhomogeneously hypoechoic lesion, (largest dimension 1.5cm) within the renal cortex which has a more hypoechoic center. On color Doppler the lesion is hypovascular with only peripheral vascularity (b).

Table 1. *Ultrasound characteristics of renal abscesses*

Patients	Location	Echogenicity	Colour doppler vascularity within lesion	Perirenal fluid	Renal stones
1	Cortex	Heterogeneously Hypoechoic	No	Yes	No
2	Cortex	Inhomogenously Hyperechoic	No	Yes	No
3	Cortex	Mixed Echogenicity	Minimal	Yes	Yes
4	Corticomedullary Junction	Hyperechoic	No	Yes	No

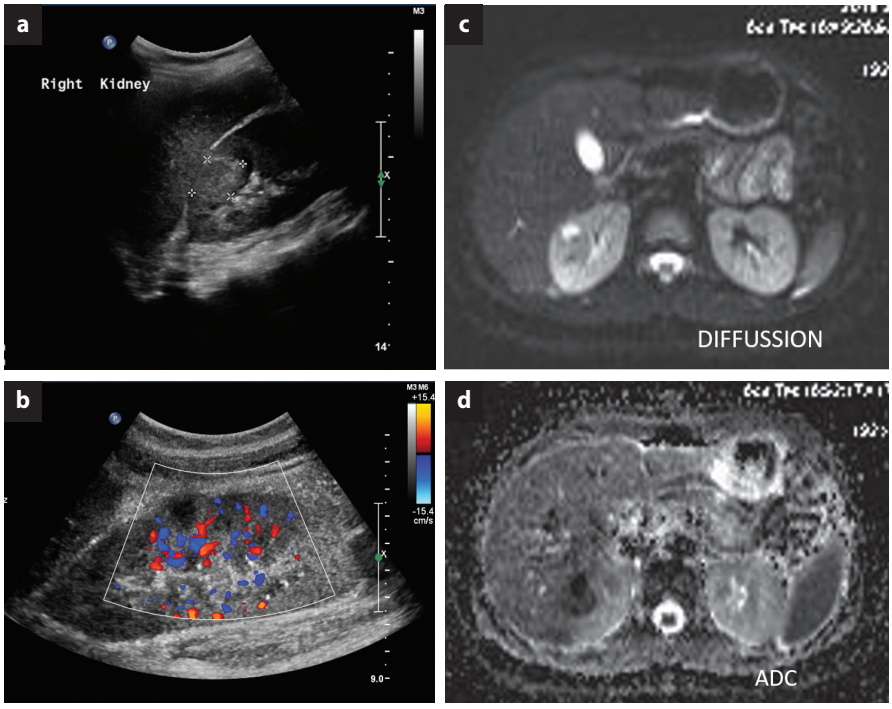


Figure 2 (a-b). Patient #2. Ultrasound images, grayscale (a) and color doppler (b) show a well-defined inhomogeneously hyperechoic lesion (largest dimension 1.5cm), within the renal cortex which has a more hypoechoic center. On color Doppler the lesion is hypovascular with peripheral vascularity only (b). **(c-d).** Patient #2. Axial DWI (c) and corresponding ADC map (d). The lesion is hyperintense on the DWI and hypointense on the ADC, consistent with true diffusion restriction.

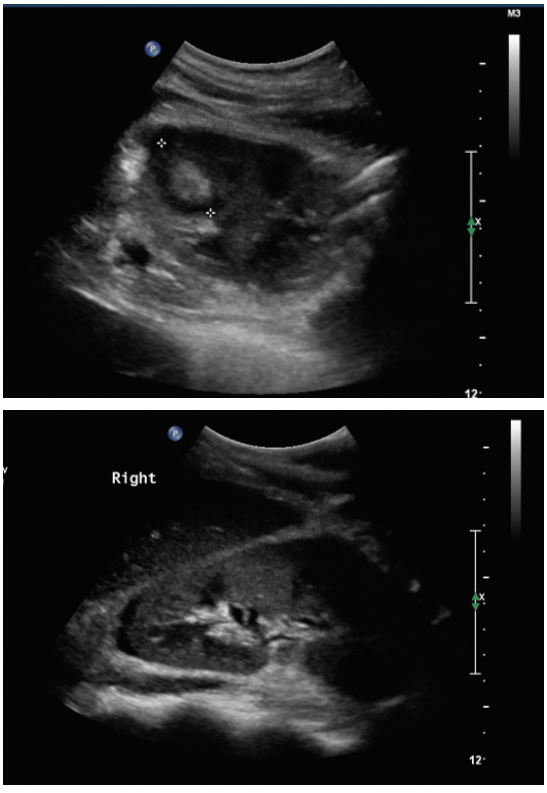


Figure 3 (a-b). Patient #3. Ultrasound grayscale images show a well-defined lesion within the renal cortex (largest diameter 1.6cm) with mixed echogenicity and predominantly hyperechoic center (a). There is also a small quantity of perirenal fluid around the upper pole of the kidney (b).

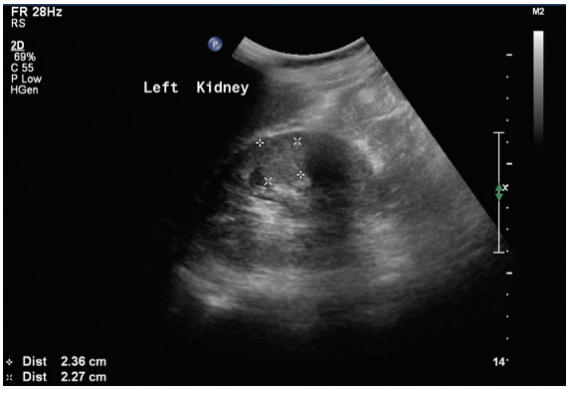


Figure 4. Patient #4. A hyperechoic lesion (largest diameter 2.4cm) centered within the corticomedullary region.

in- and opposed phase in the transverse plane. The abscess detected as an inhomogeneous lesion with internal moderately high T2-WI signal, surrounded by a thin hypointense wall. There was restricted diffusion with high signal in the DWI images and low values in the corresponding ADC map, in keeping with an abscess (Fig. 2c-d).

DISCUSSION

Renal abscesses may occasionally be present in patients with pyelonephritis as a focal or hematogenous complication [6]. The main risk factors include: diabetes

mellitus, renal calculi and ureteral obstruction. The clinical outcome of the patient relies mainly on early diagnosis, so radiological imaging plays a significant role [7]. Laboratory tests are not specific for the diagnosis of renal abscess; however, the diagnosis is established with U/S or CT/ MRI studies. U/S is the first line imaging modality performed because of its low cost, feasibility, widespread availability, and low examination time. However, CT with contrast agent outmatches US in the diagnosis of renal abscesses with a very high diagnostic rate [8]. CT finds great usage in cases of inconclusive U/S diagnoses, assessing accurate abscess size and extension and providing information about kidney function. Additionally, CT is important in the follow-up of medical treatments. On the other hand, despite the higher sensitivity and specificity of MRI and its overall diagnostic value, it is not used as a first line diagnostic technique, due to its relative inaccessibility and its higher cost. MRI is essential in patient's follow-up imaging and in cases that require avoiding radiation dose such as paediatric patients or pregnant patients. However, all the above-mentioned techniques contribute to the earliest possible diagnosis, management and follow-up of patients with renal abscesses following acute pyelonephritis [9]. A renal abscess in the ultrasonography usually presents as a well- or moderate-defined hypoechoic lesion, rarely with internal hyperechogenicity. It is usually located in the cortex or in the cortico-medullary parenchyma, regularly accompanied by perinephric collection. Septations and loculations have been described into renal abscesses. The differential diagnosis between a renal abscess and focal nephritis remains an issue for radiologists; focal nephritis usually presents with smaller lesions with poorly defined borders. The combination of acute pyelonephritis is demonstrated by a hypoechoic kidney [10]. Studies using Doppler show absence of vascularity in the necrotic pus-filled region, while the flow in the wall may be variable. A modern approach includes contrast-enhanced U/S for evaluating renal abscesses, which provides better specificity and sensitivity comparing to classic U/S. In addition, it has been supported that contrast-enhanced may be equal to CT on imaging quality, while it can be applied even in young women avoiding radiation exposure [11]. On CT, early abscess emerges as ill-defined hypoattenuating non-enhancing lesion. In the contrary, late abscess appears as well-defined hypoattenuating lesion surrounded by a thick irregular enhancing/hypervascular wall. The presence of gas within a hypoattenuating cystic formation is compatible with an abscess. Unenhanced CT is useful to provide information about calculi, gas-forming infections, haemorrhage, and parenchymal calcifications, while the use of intravenous contrast material especially the nephrographic phase (50-90 seconds) is essential for the diagnosis of renal abscess due to the significant differentiation between the normal enhancing renal parenchyma and hypovascular lesions [12,13]. Computed tomography (CT) is considered the gold standard imaging modality not only for the diagnosis and assessment of acute pyelonephritis but also for the severity of inflammation and its complications such as an abscess. However, it should not be used in young women and especially during pregnancy, due to radiation exposure concerns. MR-imaging is essential for the diagnosis of abscesses although its usage is limited. The use of diffusion-weighted images provides immediate and accurate diagnosis as it shows restriction of diffusion with low signal of the affected area, in the ADC map, which is indicative of renal abscess. Contrary to CT, it can be used for further investigation of renal abscesses especially in children and young women where radiation protection concerns are raised. [12,14]

The specificity and sensitivity of the imaging methods are not well-established with important variance, as it is affected by physicians' experience and abscess' characteristics. It is supported that newer applications of US, like tissue harmonic imaging have reported sensitivity and specificity equivalent to 97% and 80%, respectively [15]. CT provides higher sensitivity and specificity (>90%) than US, when an intravenous agent is administered [16]. The existing literature do not analyze the prognostic value of MRI for renal abscesses as a diagnostic approach.

CONCLUSION

The formation of an abscess due to pyelonephritis is very uncommon. CT is considered the gold standard examination to detect renal abscess, when clinical concern is raised, according to the literature. However, in cases of very young women of child-bearing age and/ or in cases of pregnant women ultrasound should be the first line investigation. In inconclusive cases, MRI with DWI (when available) should be used instead of CT in order to avoid radiation exposure in this sensitive group. Furthermore diffusion-weighted images can characterize the lesion without the need for IV Gadolinium administration.

Conflict of interest disclosure: *None to declare.*

Declaration of funding sources: *None to declare.*

Author contributions: *AS and PK: conception and design of the work; acquisition, analysis and interpretation of data, drafting and revising the manuscript critically for important intellectual content, and final approval of the version to be published; NN, AA and AR: conception and design of the work; acquisition, analysis and interpretation of data, drafting and revising the manuscript critically for important intellectual content; IT: drafting and revising the manuscript critically for important intellectual content.*

REFERENCES

1. Belyayeva M, Jeong JM. Acute Pyelonephritis. [Updated 2020 Jul 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK519537/
2. Craig W, Wagner B, Travis M. Pyelonephritis: radiologic-pathologic review. Radiographics. 2008;28(1):255-77; quiz 327-8.
3. Czaja CA, Scholes D, Hooton TM, Stamm WE. Population-based epidemiologic analysis of acute pyelonephritis. Clin Infect Dis. 2007; 45(3):273–280.
4. Rubilotta E, Balzarro M, Lacola V, Sarti A, Porcaro AB, Artibani, W. Current Clinical Management of Renal and Perinephric Abscesses: A Literature Review. Urologia. 2014;81(3):144-147.
5. Goel RH, Unnikrishnan R, Remer EM. Acute Urinary Tract Disorders. Radiol Clin North Am. 2015;53(6):1273–1292.
6.Grabe M, Bjerklund-Johansen TE, Botto H, Çek M, Naber KG, Pickard RS. Guidelines on Urological Infections. European Association of Urology (EAU). 2013; 4.4.2;30-1.
7. Sung BJ, Chung JM, Choi S, Rhew HY, Lee SD. Renal and Perinephric Abscesses: Ten Years of Experience at a Single Center. Korean J Urol. 2008; 49(10):923-30.
8. Coelho RF, Schneider- Monteiro ED, Mesquita JL, Mazzucchi E,Marmo Lucon A, Srougi M. Renal and perinephric abscesses: Analysis of 65 consecutive cases. World J Surg. 2007;31(2):431-6.
9. Enikeev DV, Glybochko P, Alyaev Y, Enikeev M, Rapoport L. Imaging Technologies in the Diagnosis and Treatment of Acute Pyelonephritis. Urologia. 2017;84(3):179–184.
10. Kao H-W, Wu C-J. Ultrasound of Renal Infectious Diseases. J Med Ultrasound. 2008;16(2):113–122.
11. Fontanilla T, Minaya J, Cortés C, Hernando CG, Arangüena RP, Arriaga J, et al. Acute complicated pyelonephritis: contrast-enhanced ultrasound. Abdom Imaging. 2012;37(4):639-646.
12. Colgan R, Williams M, Johnson JR. Diagnosis and treatment of acute pyelonephritis in women. Am Fam Physician. 2011;84(5):519-526.
13. Pallwein-Prettner L, Flöry D, Rotter CR, Pogner K, Syré G, Fellner C, et al. Assessment and characterisation of common renal masses with CT and MRI. Insights Imaging. 2011;2(5):543-556.
14. Chen CY, Kuo HT, Chang YJ, Wu KH, Yang WC, Wu HP. Clinical assessment of children with renal abscesses presenting to the pediatric emergency department. BMC Pediatr. 2016;16(1):1–5.
15. Lee SH, Jung HJ, Mah SY, Chung BH. Renal abscesses measuring 5 cm or less: outcome of medical treatment without therapeutic drainage. Yonsei Med J. 2010;51(4):569-573.
16. Dalla Palma L, Pozzi-Mucelli F, Ene V. Medical treatment of renal and perirenal abscesses: CT evaluation. Clin Radiol. 1999;54(12):792-797.

Corresponding author:

Aikaterini Solomou
Professor of Radiology-MRI, Head og MRI Department of MR Department, Faculty of Medicine, Department of Radiology, University Hospital of Patras, 265 04, Rio- Patras, Greece
Tel.: +30 2613 603422
E-mail: solomou@med.upatras.gr

Radiation: Health risks and precautions

Anna Mastorakou[1], Andreas Mastronikolis[2]

Abstract

Radiation is part of our daily life. Exposure to low-levels of ionizing radiation does not cause immediate health effects, but can cause a small increase in the risk of cancer over a lifetime (stochastic effect), because the human body fails to repair cells and DNA mutations. Concerning non-ionizing electromagnetic radiation, epidemiological studies demonstrated an increase in childhood leukaemia associated with exposure to magnetic field above 0.3 to 0.4 μTesla. Tissue heating is the principal mechanism of interaction between radiofrequency energy, and the human body. Epidemiological studies with 15-year follow-up of individuals show a small increase in statistical risk for brain cancer (acoustic neuroma, meningioma, glioma) in the heavy use of mobile telephony, although there are potential limitations from study design and selection bias. To date, research does not suggest any consistent evidence of adverse health effects or induction of cancer from exposure to radiofrequency fields. The risks from all appliances inside homes are greater than the risks from radiation coming from a source outside the home due to the physical weakening of the exposure on a factor of distance. Analytical research states that 30cm distance from all electrical or mobile phone devices provide a lower exposure ratio. Children and pregnant women should be protected and away from potential sources of radiation.

Key words: *Ionizing radiation; non-ionizing radiation; radon; radiofrequency radiation; 5G.*

INTRODUCTION

Online conspiracy theories linking 5G wireless services to the novel coronavirus COVID-19 outbreak had some dangerous real-world consequences. In light of recent events, the World Health Organization (WHO) and the European Commission reacted to circulating rumors and denied a possible connection between the new mobile phone generation 5G and COVID-19. WHO noted this situation as "infodemic", namely an over-abundance of information, some accurate and some not. That makes it hard for people to find trustworthy and reliable sources when they need it. While the majority of the general public might be insensitive to conspiracy theories linking 5G to COVID-19, there are enough believers to burn down infrastructure. The only way to combat conspiracy theories is education and knowledge. The purpose of this report is to provide a summary of our current understanding of radiation risks (ionizing and non-ionizing radiation) and a synopsis of recommendations and precautions in our daily life.

RADIATION

Regarding physics, radiation is the emission or transmission of energy in the form of waves or particles through space or through a material medium. The field of interest of this article is electromagnetic radiation, which is distributed in a wide spectrum of energies. Radiation is categorized as ionizing or non-ionizing depending on the energy of the emitted particles. The energy of electromagnetic radiation is determined by its frequency; ionizing radiation is high frequency and energy, whereas non-ionizing radiation is low frequency

[1]BIORMONIKH O.E., Patras, Greece
[2]School of Physics and Astronomy, University of Manchester, UK

Received: 11 Jun 2020; Accepted: 19 Oct 2020

and energy. The ionizing part of the electromagnetic spectrum includes gamma rays, X-rays and the higher energy range of ultraviolet light. In the wide range of the electromagnetic spectrum, the wave energy and frequencies met on different sources tend to increase as the wavelength shortens. Frequency is expressed in Hertz (Hz), kilohertz (KHz), megahertz (MHz), gigahertz (GHz), terahertz (THz), petahertz (PHz) and exahertz (EHz). In Figure 1, the Ionizing and non-ionizing zone of electromagnetic spectrum are pictured, with frequency and wavelength analysis.

I. IONIZING RADIATION

It is the high-energy radiation that carries more than 10 eV, which is sufficient to remove electrons during interaction with the matter and enough to ionize atoms or molecules and break chemical bonds (directly or indirectly), leading to harmful effects on the DNA of living organisms. As stated in a review article [1] published on November 2019, DNA ionization generates direct damage to the genetic macromolecule or indirect lesions due to the formation of radicals which react inevitably with the DNA constituents. Exposure to high-energy radiation, such as x-rays, gamma rays, alpha particles, beta particles, and neutrons, can damage DNA and cause cancer. These forms of radiation can be released in accidents at nuclear power plants and atomic weapons tests or disaster.

The high energy ionizing radiation comes from natural or artificial sources. About 85% of our daily life radiation is natural and 15% artificial. 40-55% of natural radiation is due to radon. Artificial radiation refers to the radioactivity produced by medical sources, and the nuclear industry. Certain medical procedures are based upon ionizing radiation [x-rays, radiation therapy, computed tomography (CT) scans, positron emission tomography (PET) scintigraphy, etc] and can also cause cell damage that leads to cancer. However, the risks of cancer from these medical procedures are very small as compared to the benefit from having them in some medical conditions.

Expressed in Sievert (unit of measurement of the biological effect in the human body by ionizing radiation levels), the whole-body population limit exposure is 1mSv / year. Average environmental exposure to radiation is about 3mSv / year. It is crucial for public to understand the radiation protection principles of minimal time exposure, distance and shielding. The basic rule of exposure to ionizing radiation is A.L.A.R.A. (As Low As Reasonable Achievable).

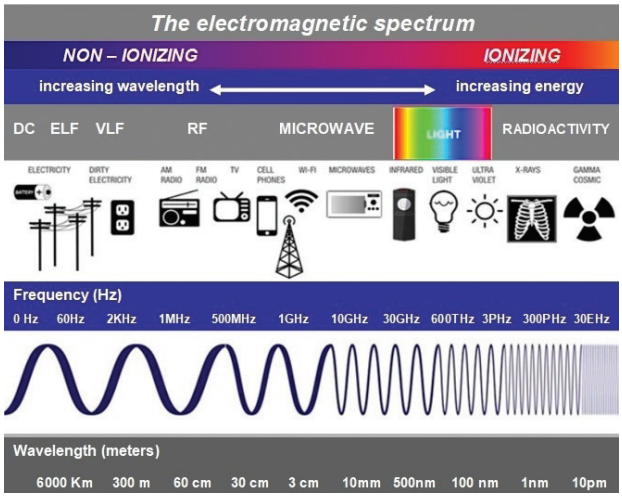


Figure 1. The electromagnetic spectrum: Ionizing and non-ionizing radiation. Different types of electromagnetic waves, frequencies, and energy bands.
Abbreviations: DC: Direct Currents; ELF: Extremely Low Frequency; VLF: Very Low Frequency; RF: Radio Frequency.

I.A. Radon: The invisible threat to homes

Radon ($_{86}$Rn) is the world's most important source of exposure to ionizing radiation. Radon is a radioactive, colorless, odorless, tasteless noble gas, which is the immediate decay product of radium (^{226}Ra), derived as a decay product of uranium (^{235}U). The isotope ^{222}Rn has a half-life of 3.8 days. Radon was classified as a human carcinogen in 1988, by IARC (International Agency for Research on Cancer), a branch of WHO. ^{222}Radon is especially dangerous because it permeates soil and rocks and concentrates inside buildings and uranium mines. Radon was identified as a health problem by the fact of observed high rates of lung cancer in underground uranium miners who were exposed to it. At high concentrations, gaseous ^{222}Rn may be inhaled and decay to radioactive polonium (^{210}Po) in the lungs, whose high-energy alpha radiation bombards vulnerable lung tissue and damages cells. Alpha radiation is produced by alpha particles, which consist of two protons and two neutrons, structurally identical to the nucleus of the gas helium. Alpha radiation is a highly ionizing form of particle radiation with a low penetration depth that irradiates tissues, causing biological damage from chromosome alterations and mutations.

Extended periods of exposure to ^{222}Rn and its progeny ultimately induce lung cancer, as is stated by WHO [2] and EPA (U.S. Environment Protection Agency). According to WHO, the proportion of all lung cancers linked

to radon is estimated to be 3% to 14%, depending on the average radon concentration status. The majority of radon-induced lung cancers are caused by low and moderate radon concentrations. Alternatively, radon may enter the body through contaminated drinking water, making radon diffusion one of the greatest dangers of radium. Thus, ^{222}Rn is a carcinogen; in fact, Radon is the second most important cause of lung cancer after cigarette smoking in many countries [3, 4] and in the United States, with over 20,000 deaths per year attributed to radon-induced lung cancer, according to EPA. Similar findings are found worldwide. Residential radon exposure is considered as the first cause of lung cancer in never smokers [5, 6]. Exposure to the combination of radon gas and cigarette smoke creates a greater risk of lung cancer. WHO stated that radon exposure is a major and growing public health threat in homes and recommends that countries adopt reference gas levels of 100 Bq/m^3 (Becquerel per cubic meter).

Radon is a pollutant that affects the quality of indoor air worldwide. It typically moves upwards through the ground and into the house through cracks and other holes in the foundation (cracks in floors, walls, construction joints, around service pipes, etc). Radon gas enters the house being trapped inside, especially in the winter months, and in the night, when windows and doors are kept closed. It is also found in thermal springs, caves, mines, underground workplaces and well water. The Greek Atomic Energy Commission (E.E.A.E.) has much useful information about radon and an application in its website that provides access to information about radon concentrations (Bq/m^3) in different regions of Greece. Measurements of the indoor radon level of a home are accomplished by request to the Commission.

I.B. Radioactive polonium: invisible threat inside tobacco

Smoke contains radioactive Polonium (^{210}Po) with a half-life of T1/2 = 138.4 days. Polonium has been found in smoke from tobacco leaves grown with phosphate fertilizers, which contains radium (^{226}Ra), ancestor of ^{210}Po. ^{210}Po is an important contaminant in the environment, mainly affecting seafood and tobacco. As a result of its intense radioactivity, it is extremely toxic to humans. During smoking, ^{210}Po is absorbed by the bronchial system, and the lungs are exposed to alpha radiation along with other toxins, creating a toxic / carcinogenic mixture.

There are various studies confirming the radiological risk from ^{210}Po in a smoker of 20 cigarettes per day for a year. The risk is equivalent to the one deriving from 300 chest X-rays, with an autonomous oncogenic capability of 4 lung cancers per 10.000 smokers. ^{210}Po can also be found in passive smoke surrounding environment [7]. The U.S. International Commission on Radiation Protection (ICRP) estimates of the lifetime probability of developing lung cancer after a 1-Sv radiation dose, is 121 cases per 100 000 population (1.21%) [8].

I.C. Artificial tanning carries a risk of skin cancer

Ultraviolet radiation (UVR) is a natural part of solar radiation with wavelength from 10nm to 400nm, and is released by tanning beds, black lights, and electric arc lighting. Artificial tanning light bulbs emit UVR, which is found at the borderline zone of ionizing and non-ionizing radiation in the electromagnetic spectrum. Modern light bulbs emit ultraviolet A (UVA) and ultraviolet B (UVB) radiation, but UVA intensity can be up to 15 times stronger than solar radiation. In 2009, IARC has described ultraviolet radiation (solar and artificial), as "carcinogenic" to humans, because it causes skin cancer and melanoma. Exposure to UVR from artificial tanning beds devices leads to DNA damage in skin cells and is associated with an increased risk of developing basal / squamous cell carcinomas, and melanoma [9-11]. It has also been associated with skin burns, accelerated skin aging, ocular melanoma and immune suppression. Consistent regulation of the intensity, and UV wavelength emitted by indoor tanning devices is crucial. The use of a tanning facility in early adulthood has been strongly associated to high risk of skin cancer. Elevated vitamin D levels associated with UV exposure have been suggested to protect against various internal malignancies and other diseases, but other sources of vitamin D are easily available.

Artificial tanning devices exert their effect through the emission of both UVA and UVB radiation. While UVB is associated with direct DNA damages by the photoproducts, UVA exposure is associated with indirect DNA damages through the production of reactive oxygen species. While indoor tanning remains prevalent in population, it is clear that artificial tanning bed represents an avoidable risk for skin cancer and a widespread public health issue.

I.D. Medical diagnostic and therapeutic procedures

Even though the use of ionizing radiation in medicine poses potential risks to patients, medical personnel, and

the general public, however, it also offers enormous benefits in selected cases. Modern medicine would be impossible without the use of ionizing radiation. As seen below in Table 1, radiation doses variability is high, depending on the diagnostic medical procedure.

TYPICAL RADIATION DOSES FROM COMMON MEDICAL IMAGING PROCEDURES

In recent decades, for dose limitation purposes, the International Commission on Radiation Protection (ICRP) has divided the diverse radiation effects into either stochastic effects (cumulative low dose effect with no apparent threshold) or tissue reactions (formerly termed non-stochastic or deterministic effects, which do have a threshold of visible injury). It is known that cancer risks increase almost linearly as exposure doses increase above approximately 150 mSv. Relying on the uncertainty of the stochastic risk from ionizing radiation associated with medical imaging, it is impossible to describe precisely the risks of a medical imaging procedure. The estimated risk from a diagnostic or therapeutic medical procedure should be compared to general statistical risks of death from other common causes.

Table 1. *The radiation exposure is high in several techniques of cardiology imaging. Source: Greek Atomic Energy Commission (E.E.A.E.).*

Medical procedure	Effective dose in Millisieverts (mSv)
Dental X-Ray	0.005
Upper / lower extremity X-Ray	0.001
Chest X-Ray	0.02
Skull X-Ray	0.03
Mammogram	0.3
Lumbar spine X-Ray	1.0
Intravenous urography	3.0
Computer Tomography (CT) of the head / brain	2.4
CT of the chest	9.0
CT of the abdomen and pelvis	18.0
Coronary angiography	14.0
Percutaneous transluminal coronary angioplasty	31.0
Pacemaker implant	4.0
Nuclear bone scan with Technetium 99m (99mTc)	3.8
Nuclear medicine myocardium stress test with 99mTc	2.5
Nuclear medicine myocardium stress test – Thallium201	15.0
Positron Emission Tomography (PET) / CT	6.6

In the late 1990s, data was released by the Radiation Effects Research Foundation of their longitudinal study of 50.000 survivors of the atomic bombs in Hiroshima and Nagasaki who were exposed to radiation doses of less than 500 mSv. Analysis of solid cancer incidence in these individuals was performed after a follow up over 55 years [12]. The investigators found direct and statically significant evidence of risk in the dose range from 50–100 mSv. This linear no-threshold hypothesis is questioned by a strong biologic argument. With doses up to 50 mSv, error-free DNA repair is expected. With doses over 100 mSv, error-prone repair is possible. At this level, some aberrant cells may go on to become preneoplastic cells that may then differentiate into invasive cancers. Tubiana et al. [13] concluded there is no evidence of a carcinogenic effect for acute radiation doses less than 100 mSv. The International Atomic Energy Agency (IAEA) has adopted a protection strategy with reference level of 20-100mSv, in order to reduce the risks of stochastic effect. General principles of radiation protection from the hazard of ionizing radiation in medicine are summarized in three principles: justification of the procedure, optimization of individual protection, and dose limitation. The best approach on stochastic effects is suggested by the consensus between the physician who has the responsibility of evaluating the relevant risks from a medical procedure and the patient who has to accept those risks as possible outcomes in a written informed consent [14].

II. NON-IONIZING RADIATION

Electromagnetic fields (EMF) are fields of energy produced by moving electrical charges. In the electromagnetic fields, the oscillations of electric and magnetic fields can propagate in space in the form of a wave and transmit energy at the speed of light. Electromagnetic radiation is defined according to its wavelength and frequency, which is defined as the number of cycles of a wave from a reference point per second. Its frequency is measured in Hz. Non-ionizing radiation

sources include power lines, radio waves, microwaves, lasers, infrared radiation, and visible light. **Non-ionizing electromagnetic radiation** is a relatively low-energy radiation that is not able to ionize atoms or molecules but can produce kinetic energy which is converted to heat. This heat can adversely affect health in a range of ways. Non-ionizing radiation has sufficient energy for excitation (movement of an electron to a higher energy state), nerve stimulation (from 100 kHz to 10 MHz, adverse health effects (from 10MHz to 300 GHz), disturbance of the electrochemical balance of the cell membrane, and thermal effect.

Extremely low frequency (ELF) radiation is the radiation produced by structure like power lines or electrical wiring.

Radiofrequency (RF) and microwave (MV) radiation. Electromagnetic fields in the radiofrequency range are used for telecommunications applications, including cell phones, televisions, and radio transmissions. The human body absorbed energy from these devices, is estimated by a measure called the specific absorption rate (SAR), which is expressed in watts per kilogram of body weight. The effects of non-ionizing radiation depend on the intensity, frequency and degree of exposure. Although considered less dangerous than ionizing radiation, overexposure to non-ionizing radiation can cause public health issues.

II.A. Extremely low frequency (ELF) radiation

Electric fields arisen from electric charges, are measured in volts per meter (V/m). **Magnetic fields** arisen from the motion of electric charges are expressed in tesla (T), millitesla (mT) or microtesla (μT). Electric fields are produced independently of the device function, whereas magnetic fields are produced only when a current is flowing, and the device is functioning. Power lines produce magnetic fields continuously, due to current flowing through them. Electric fields are easily shielded or weakened by the walls, wood, metal and other objects, whereas magnetic fields can pass through buildings, living things, and most other materials. In the past years there is a public health concern and debate about daily exposure to extremely low-frequency magnetic fields (ELF-EMF) as possibly harmful to human health (cancer, neurobehavioral disturbances, etc). Pooled analyses of epidemiological studies suggested a pattern of an increase in childhood leukaemia associated with exposure to residential power magnetic field above 0.3 to 0.4 μT [15, 16]. This has resulted in the classification of ELF-EMF into category 2B, i.e., agents that are "possibly carcinogenic to humans" by the International Agency for Research on Cancer. The reference limit for electric fields set by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) is 5KV/m. Exposure to electric field from home devices is far below this limit.

Table 2. *Magnetic field strength around all appliances rapidly decreases with the distance. Normal operation distance is given in bold. At a distance of 30 cm the magnetic fields surrounding most household appliances are much lower than the given guideline limit of 100 μT for the general public (WHO). Source: WHO /Federal Office of Radiation Safety, Germany 1999.*

Electrical appliance	3cm distance (μT)	30cm distance (μT)	1m distance (μT)
Hair dryer	6 - 2000	0.01 - 7	0.01 - 0.03
Electric shaver	16 - 1600	0.08 - 9	0.01 - 0.03
Vacuum cleaner	200 - 800	2 - 20	0.13 - 2
Fluorescent light	40 - 400	0.5 - 2	0.02 - 0.25
Microwave oven	73 - 200	4 - 8	0.25 - 0.6
Portable radio	16 - 56	1	< 0.01
Electric oven	1 - 50	0.15 - 0.5	0.01 - 0.04
Washing machine	0.8 - 50	0.15 - 3	0.01 - 0.15
Iron	8 - 30	0.12 - 0.3	0.01 - 0.03
Dishwasher	3.5 - 20	0.6 - 3	0.07 - 0.3
Computer	0.5 - 30	< 0.01	
Refrigerator	0.5 - 1.7	0.01 - 0.25	< 0.01
Colour TV	2.5 - 50	0.04 - 2	0.01 – 0.15

The overall exposure to electromagnetic radiation depends on the strength of the electromagnetic field, the distance from the source of the field, and the length of exposure time. The highest exposure occurs when there is short distance to a strong field for a long period. Table 2 shows magnetic field (μTesla) derived from different house devices depending on the distance from the source.

TYPICAL MAGNETIC FIELD STRENGTH OF HOUSEHOLD APPLIANCES AT VARIOUS DISTANCES

II.B. Radio frequency (RF)

Radiofrequency (RF) electromagnetic radiation (EMR) is the transfer of energy by radio waves. The frequency of radiofrequency electromagnetic radiation ranges from 30 kilohertz (30 kHz) to 300 gigahertz (300 GHz). Relatively high levels of exposure to RF fields can occur to workers in the broadcasting, transport, and communications industries when workplace is in proximity to RF transmitting antennas. Overall, the RF field background level from household appliances is low.

Mobile phones are low-powered radiofrequency transmitters, operating at frequencies between 450 and 2,700 MHz. The handset only transmits power when it is turned on. The power and the radiofrequency exposure fall off rapidly with the increasing distance from the handset. A person **using a mobile phone 30–40 cm away from their body** will have a much lower exposure to radiofrequency fields compared to the contact point. "Hands-free" devices will reduce exposure keeping mobile phones distant from the head and body during phone calls. Limiting the number and length of calls is also a best practice.

Tissue heating is the principal interaction mechanism between radiofrequency energy, and the human body. In the case of frequencies used by mobile phones, most of the energy is absorbed by the skin, and superficial tissues, resulting in negligible temperature rise in the contact point of the body. In relation to mobile phone exposure, the SAR distribution in the head depends on many factors including head size, frequency, and exposure conditions. Thus, compared to adults, higher SARs is expected to occur in children's brain because of thinner skin, and surrounding tissue [17].

The strength of the signal transmitted between a mobile phone and base station would vary by distance between both antennas, according to the inverse square law. The use of Adaptive Power Control (APC) technique in mobile phones enables customization of output powers, so that, the signal strength and synchronization to base stations is constant and sufficient to produce good quality reception. The maximum SAR depends critically on the position of the phone and, in particular, on the distance between the antenna and the brain. Using the phone in areas of good reception also decreases exposure to the operator, as it allows cell phone to transmit at reduced power.

Several large multinational epidemiological studies have ended or are ongoing, including case-control studies and prospective cohort studies. Epidemiological studies with a 15-year follow-up of individuals show a small increase in statistical risk for brain cancer (acoustic neuroma, meningioma, glioma) in the heavy use of mobile telephony [18], although there are potential limitations from study design and selection bias.

The largest retrospective case-control study to date on adults, Interphone [18], coordinated by IARC, was designed to determine whether there are links between the use of mobile phones and brain cancers in adults. The international pooled data analysis gathered from 13 participating countries found no increased risk of glioma or meningioma with mobile phone use of more than 10 years. There are some indications of an increased risk of glioma for those who reported the highest 10% of cumulative hours of cell phone use. The researchers concluded that biases and errors limit the strength of these conclusions. Moreover, no association or possible indication between heavy mobile phone use and brain tumours, were concluded on Danish study [19], Million Women Study [20], Cerenat study [21] and Cefalo study [22]. IARC has found limited evidence that RF radiation causes cancer in animals and humans, and classified RF radiation as "possibly carcinogenic to humans" (Group 2B). This was based on the finding of a possible link in at least one study between cell phone use, and a specific type of brain tumour. IARC considers the evidence overall to be limited and inconclusive because of the methodologic limitations and conflicting findings in some studies. The U.S. Food and Drug Administration (FDA) stated that based on the current information, the current safety limits for cell phones are acceptable for protecting the public health. A large prospective cohort study of cell phone use, and its possible long-term health effects was launched in Europe in March 2010. This ongoing study, known as COSMOS, has enrolled approximately 290,000 cell phone users' aged 18 years or older to date and will follow them for 20 to 30 years [23][24].

A number of studies have investigated the effects

of radiofrequency fields on heart rate, brain electrical activity, sleep, cognitive function, and blood pressure in volunteers. To date, research does not suggest any consistent evidence of adverse health effects from exposure to radiofrequency fields. Further, research has not been able to provide support for a relationship between exposure to electromagnetic fields and self-reported symptoms of "electromagnetic hypersensitivity" [25].

II.C. Wireless local area networks

Most Wi-Fi devices operate at radiofrequencies that are broadly similar to cell phones, typically 2.4 - 2.5 GHz, although, in recent years Wi-Fi devices with higher frequencies (up to 5.8 GHz) have appeared. Radiofrequency radiation exposure from Wi-Fi devices is considerably lower than that from cell phones. Both sources emit levels of radiofrequency radiation that are far below the guideline of 10 W/m2 as specified by the ICNIRP. According to a 2019 review, there is no noticeable increase in everyday EMF exposure since 2012, despite the increasing use of wireless communication devices [26].

II.D. 5G

5G is the 5th generation of mobile networks. Due to the increased bandwidth and the higher frequency of the new network, the download speed is expected to reach up to 10 Gigabits/sec. In Figure 2, 5G band is showed in electromagnetic spectrum as compared to existing cellphones technology.

5G technology uses millimetre waves (MMW), in addition to the microwaves that have been used to date in previous "2G, 3G, 4G" networks. The increased frequency of 5G does not necessarily mean increased user exposure, as this depends on the distance, duration of exposure and the degree of attenuation of the radiation.

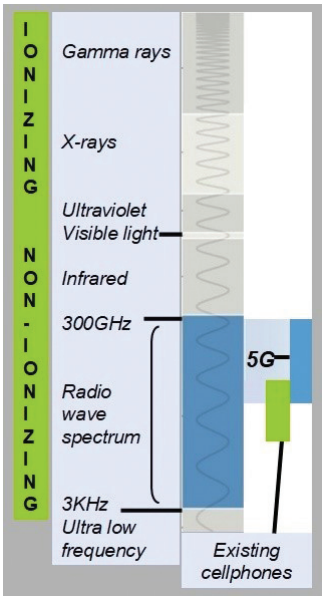


Figure 2. 5G electromagnetic spectrum. 5G operation non-ionizing band is compared to zone occupied by existing cellphones. Due to the short wavelengths, 5G does not penetrate objects and human skin, and cannot travel long distances, hence 5G technology requires a larger number of base stations than 4G.

Regarding physical characteristics, the non-ionizing high-frequency electromagnetic radiation of 5G has a small penetration range in the body. The skin acts as a barrier shielding the internal organs from exposure to 5G radiation. This is a fact also for sunlight and ultraviolet light which have a higher frequency than 5G. Furthermore, there is a significant weakening of 5G radiation from building materials, which explains why a denser network of base stations is required for the acquirement of stable signal.

In recent years, a growing appeal [27] from hundreds of scientists worldwide states that the increasingly extensive use of wireless technology, especially with the use of 5G technology, would expose population to constant EMF radiation because of the large number of 5G transmission stations suitable for billions of connections. In addition, the appeal points to a large number of scientific publications that illustrate EMF harmful exposure effects. They urge "the EU to follow Resolution 1815 of the Council of Europe and demand a new assessment by an independent task force".

The 5G radio emission fields are quite different to those of previous generations because of their complex beamformed transmissions which produce a focused wireless signal towards a specific receiving device. The beam modifies vary rapidly with time and movement and so are unpredictable, as the signal levels and patterns seem to interact as a closed loop system. This has yet to be mapped reliably for real world situation, outside laboratory conditions. Along with the mode and duration of exposures, characteristics of the 5G dense signal pulse seem to increase the biologic and health impacts of exposure [28]. A 2018 review concludes that "evidence of the biological properties of radiofrequency EMF (including 5G) are accumulating progressively and preliminary studies point to the existence of multi-level interactions between high-frequency EMF and biological systems" [29].

Data from a pragmatic review that analyzed 94 relevant publications performing in vivo or in vitro investigations about the health impact of MMW, led to the conclusion that "regarding the health effects of MMW in the 6–100 GHz frequency range at power densities not

exceeding the exposure guidelines the studies provide no clear evidence, due to contradictory information from the in vivo and in vitro investigations" [30].

On March 2020, the European Parliament Research Service (EPRS), by author Karaboytcheva M, issued a briefing about "Effects of 5G wireless communication on human health". According to the 2019 European Parliament study "5G deployment: State of Play in Europe, USA and Asia", long-term technology research is essential. One key problem is the unusual propagation phenomena, especially measuring radio frequency EMF exposure at MMW frequencies for the handset and the base station. The study states that the main problem seems to be that it is not currently possible to accurately simulate or measure 5G emissions in the real world.

In a recent statement named "Scientific Evidence for Cell Phone Safety", FDA declared no new implications for the band of 5G maintaining the existing exposure limits and guidelines. The ICNIRP RF EMF guidelines 2020 has made a number of changes to ensure that new technologies such as 5G will not be able to cause harm. "These changes include the addition of whole-body average restrictions for frequencies >6 GHz, restrictions for brief (<6 minutes) exposures and the reduction of the averaging area for frequencies >6 GHz". The Australian Radiation Protection and Nuclear Safety Agency (ARPANSA) stated that "the operating frequencies of the 5G network are considered within the limits and no health effects are expected from radio frequency exposures below the limits set in the ARPANSA standard".

III. PROTECTION AND PRECAUTIONS INSTRUCTIONS TO PUBLIC

- To protect public against exposure to radon, it is necessary to check and isolate the foundation of the buildings and ventilate the interior environment so the gas escapes outside and the exposure inside the house or workplace is reduced. Radon measurement is carried out by request to the Hellenic Atomic Energy Commission. Smoking should be stopped inside home.
- The full implementation of the anti-smoking law is mandatory to avoid exposure to the radioactive polonium (210Po) contained in cigarette smoke, especially in the case of passive smoking indoors. Special attention must be given to young children and pregnant women.
- People should avoid exposure to artificial light devices - artificial tanning as strong exposure to ultraviolet radiation is classified as carcinogenic.
- General principles of radiation protection from the hazard of ionizing radiation and the safety issues by medical radiological or nuclear medicine equipment are summarized in three principles: justification of the procedure, optimization of individual protection, and dose limit [31, 32]. In radiation therapy, the predominant issue is the avoidance of accidents.
- It is recommended to restrict time using mobile phones and to remove the device far away from the user's head and body (hands-free, Bluetooth, etc.). The safety distance must be greater than 30-40 cm [33].
- In areas with bad radiofrequency signal, the exposure to operator increases, because mobile phone emits with increased power levels. It is recommended to avoid using a mobile phone in basements, enclosed spaces, elevators and on a vehicle. Metal mobile phone cover should be avoided because it leads to mobile phone operation status with maximum power.
- Mobile phone should be put in flight mode whenever is possible and especially in the case of children dealing with games [33].
- Schools and homes should give preference to wired internet connections and regulate strictly the use of mobile phones by the children.
- The wi-fi device or other devices should not be near a bed or desk where the user's exposure may be long. Devices that are not used at night should be turned off [33].
- The use of electric blanket should be avoided during sleep. Sleeping close to iron materials is not recommended [33].
- Safety distances must be kept from electrical appliances to 30 cm to weaken the exposure to electromagnetic radiation [33].
- The Hellenic Atomic Energy Commission is responsible for the installation, and operation of mobile telephony and radio frequency antennas. There are annual reports for compliance to security limits, mapping of mobile phone antenna measurements nationwide and information material on the EEAE website (https://eeae.gr/).

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare.*

Author contributions: *Anna Mastorakou: conception, design, drafting of the article; critical revision of the article for*

important intellectual content; final approval of the article and Andreas Mastronikolis: analysis and interpretation of physical data; final approval of the article.

REFERENCES

1. Mavragani IV, Nikitaki Z, Kalospyros SA, Georgakilas AG. Ionizing Radiation and Complex DNA Damage: From Prediction to Detection Challenges and Biological Significance. Cancers (Basel). 2019;11(11):1789.
2. Ting D. WHO Handbook on Indoor Radon: A Public Health Perspective. International Journal of Environmental Studies. 2010;67(1):100-102.
3. Darby S, Hill D, Auvinen A, Barros-Dios JM, Baysson H, Bochicchio F, et al. Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005;330(7485):223.
4. Gaskin J, Coyle D, Whyte J, Krewksi D. Global Estimate of Lung Cancer Mortality Attributable to Residential Radon. Environ Health Perspect. 2018;126(5):057009.
5. Rodríguez-Martínez Á, Torres-Durán M, Barros-Dios JM, Ruano-Ravina A. Residential radon and small cell lung cancer. A systematic review. Cancer Letters. 2018;426:57–62.
6. Torres-Durán M, Ruano-Ravina A, Parente-Lamelas I, Leiro-Fernández V, Abal-Arca J., Montero-Martínez C, et al. Residential Radon and Lung Cancer Characteristics in Never Smokers. Int J Radiat Biol. 2015;91(8):605-10.
7. Zagà V, Lygidakis C, Chaouachi K, Gattavecchia E. Polonium and Lung Cancer. J Oncol. 2011; 2011:860103.
8. Karagueuzian HS, White C, Sayre J, Norman A. Cigarette Smoke Radioactivity and Lung Cancer Risk. Nicotine Tob Res.2012;14(1):79-90.
9. Schulman JM, Fisher DE. Indoor ultraviolet tanning and skin cancer: health risks and opportunities. Curr Opin. 2009;21(2):144–149.
10. Le Clair MZ, Cockburn MG. Tanning bed use and melanoma: Establishing risk and improving prevention interventions. Prev Med Rep. 2016;3:139–144.
11. Boniol M, Autier P, Boyle P, Gandini S. Cutaneous melanoma attributable to sunbed use: systematic review and meta-analysis. BMJ. 2012;345:e4757.
12. Pierce DA, Preston DL. Radiation-related cancer risks at low doses among atomic bomb survivors. Radiat Res. 2000;154(2):178–186.
13. Tubiana M, Feinendegen LE, Yang C, Kaminski JM. The Linear No-Threshold Relationship Is Inconsistent with Radiation Biologic and Experimental Data. Radiology. 2009;251(1):13–22.
14. Brink JA, Goske MJ, Patti JA. Informed Decision Making Trumps Informed Consent for Medical Imaging with Ionizing Radiation. Radiology. 2012;262(1):11–14.
15. Kheifets L, Ahlbom A, Crespi CM, Draper G, Hagihara J, Lowenthal RM, et al. Pooled analysis of recent studies on magnetic fields and childhood leukaemia. Br J Cancer.2010;103(7):1128–1135.
16. Amoon AT, Crespi CM, Ahlbom A, Bhatnagar M, Bray I, Bunch KJ, et al. Proximity to overhead power lines and childhood leukaemia: an international pooled analysis. Br J Cancer. 2018;119(3):364-373.
17. Cardis E, Deltour I, Mann S, Moissonnier M, Taki M, Varsier N, et al. Distribution of RF energy emitted by mobile phones in anatomical structures of the brain. Phys Med Biol. 2008;53(11):2771-2783.
18. Swerdlow AJ, Feychting M, Green AC, Kheifets L, Savitz DA. Mobile Phones, Brain Tumors, and the Interphone Study: Where Are We Now? Environ Health Perspect. 2011;119(11):1534–1538.
19. Frei P, Poulsen AH, Johansen C, Olsen JH, Steding-Jessen M, Schuz J. Use of mobile phones and risk of brain tumours: update of Danish cohort study. BMJ. 2011;343:d6387.
20. Benson VS, Pirie K, Schüz J, Reeves GK, Beral V, Green J, et al. Mobile phone use and risk of brain neoplasms and other cancers: prospective study. Int J Epidemiol. 2013;42(3):792-802.
21. Coureau G, Bouvier G, Lebailly P, Fabbro-Peray P, Gruber A, Leffondre K, et al. Mobile phone use and brain tumours in the CERENAT case-control study. Occup Environ Med. 2014;71(7):514-522.
22. Aydin D, Feychting M, Schüz J, Tynes T, Andersen TV, Schmidt LS, et al. Mobile phone use and brain tumors in children and adolescents: a multicenter case-control study. J Natl Cancer Inst. 2011;103(16):1264-1276.
23. Schüz J, Elliott P, Auvinen A, Kromhout H, Poulsen AH, Johansen C, et al. An international prospective cohort study of mobile phone users and health (Cosmos): design considerations and enrolment. Cancer Epidemiol. 2011;35(1): 37-43.
24. Toledano MB, Auvinen A, Tettamanti G, Cao Y, Feychting M, Ahlborn A, et al. An international prospective cohort study of mobile phone users and health (COSMOS): Factors affecting validity of self-reported mobile phone use. Int J Hyg Environ Health. 2018;221(1):1-8.
25. (ICNIRP) Guidelines for Limiting Exposure to Electromagnetic Fields (100 kHz to 300 GHz). Health Phys. 2020;118(5):483–524.
26. Jalilian H, Eeftens M, Ziaei M, & Röösli M. Public exposure to radiofrequency electromagnetic fields in everyday microenvironments: An updated systematic review for Europe. Environ Res. 2019;176:108517.
27. Hardell L, Nyberg R. Appeals that matter or not on a moratorium on the deployment of the fifth generation, 5G, for microwave radiation. Mol Clin Oncol. 2020;12(3):247-257.
28. Kostoff RN, Heroux P, Aschner M, Tsatsakis A. Adverse health effects of 5G mobile networking technology under real-life conditions. Toxicol Lett. 2020;323:35-40.
29. Di Ciaula A. Towards 5G communication systems: Are there health implications? Int J Hyg Environ Health. 2018;221(3):367–375.
30. Simkó M, Mattsson MO. 5G Wireless Communication and Health Effects—A Pragmatic Review Based on Available

Studies Regarding 6 to 100 GHz. Int J Hyg Environ Health. 2019;16(18):3406.
31. ICRP Publication 105. Radiation protection in medicine. Ann ICRP. 2007;37(6):1-63.
32. Do KH. General Principles of Radiation Protection in Fields of Diagnostic Medical Exposure. J Korean Med Sci. 2016;31 Suppl 1(Suppl 1):S6-S9.
33. Belyaev I, Dean A, Eger H, Hubmann G, Jandrisovits R, Kern M, et al. EUROPAEM EMF Guideline 2016 for the prevention, diagnosis and treatment of EMF-related health problems and illnesses. Rev Environ Health. 2016;31(3):363-397.

Corresponding author:

Anna Mastorakou,
Korinthou 210-212, 26221, Patras
Tel.: +30 2610 275556, fax: 2610243725
E-mail: anima@otenet.gr

Feeding and Swallowing Disorders in the Paediatric Population. The Speech and Language Pathologist's Perspective

Angeliki Zarkada[1,2], Rigas Dimakopoulos[2,3], Terpsithea Germani[1], Angeliki Mantzari[2], Georgia Karadimou[2]

Abstract

The development of feeding and swallowing skills is a complex process, which begins during the prenatal period and continues until early childhood. Feeding and swallowing disorders (dysphagia) may occur in the paediatric population, affecting the oral phase, the triggering of the swallowing reflex, the pharyngeal phase and the oesophageal phase of swallowing. Paediatric dysphagia may present with various symptoms relevant to the phases of swallowing, and it is associated with several conditions, including medical, nutritional, psychosocial disturbances and impaired feeding skills. Speech and language pathologists (SLPs) play a key role in the assessment and management of paediatric dysphagia. The fact that paediatric dysphagia is multifactorial, makes the contribution of a multidisciplinary team to the assessment and management of feeding and swallowing dysfunction imperative. A comprehensive assessment of the paediatric population with dysphagia includes clinical and instrumental evaluation, while paediatric dysphagia management involves a range of strategies, which have been developed to ensure the efficiency and safety of feeding and swallowing process and improve children's quality of life (QoL).

Key words: *Paediatric dysphagia; assessment; management; speech and language pathologist*

INTRODUCTION

It is estimated that around 20-45% of infants and young children exhibit some form of feeding and swallowing difficulty [1-3] , demonstrating various complications, such as slow weight gain, disrupted nutrition and acute choking. Severe complications relevant to feeding and swallowing disorders may lead to aspiration-based infections of the nasal sinuses, middle ears and lungs, which may threaten children's life [4-7]. On the strength of medical and technological development, neonatal and infant mortality has significantly decreased leading to a rise in the number of children with feeding and swallowing disorders, and proper care focuses not only on survival but also on quality of life (QoL) improvement. For this reason, the range and complexity of feeding and swallowing disorders challenges healthcare, educational and rehabilitation systems [8]. The complete understanding of both normal and impaired feeding and swallowing process is integral for speech and language

[1]ELEPAP, Rehabilitation for the Disabled, Athens, Greece
[2]"Motivaction". Rehabilitation center for children with neurodevelopmental disabilities, Athens, Greece
[3]"Department of Physiotherapy, Faculty of Health & Caring Professions, University of West Attica, Athens, Greece

Received: 13 May 2020; Accepted: 11 Jul 2020

pathologists (SLPs) to carry out a comprehensive feeding and swallowing assessment and to make optimal management decisions for every child, dealing with medical, nutritional, psychosocial issues and feeding dysfunction. Although SLPs play a central role in the assessment and management of dysphagia, children and their parents/ caregivers are better served by a multidisciplinary team [9].

The primary objective of the current review is to summarize most recent evidence-based data related to paediatric feeding and swallowing disorders. The secondary aim is to bring up to date the paediatric specialists about different topics related to paediatric dysphagia, to familiarize them with signs of dysphagia and inform them under what circumstances they should refer these children to an SLP. Awareness of all topics related to paediatric dysphagia is crucial for SLPs and paediatric specialists to conduct appropriate evaluations and therapeutic procedures for every child.

THE PROCESS OF NORMAL SWALLOWING

Swallowing is a multidimensional complex procedure, which requires intact innervation of the muscles related to swallowing, structural integrity of the musculoskeletal systems of the head, neck, thorax and abdomen, adequate airway protection and intact gastrointestinal system. The process of swallowing involves four phases, including the (i) oral phase, (ii) triggering of the swallowing reflex, (iii) pharyngeal phase and (iv) oesophageal phase [10].

Several studies describe the physiology of normal eating and swallowing procedure, suggesting that this process involves volitional and reflexive activities [10, 11]. The oral phase of swallowing combines the oral preparation of the food/ fluid bolus (oral preparatory phase) and the oral propulsion of the food/ fluid bolus (oral propulsive phase). In neonates and young infants, the oral phase is reflexive and involuntary. However, later in infancy, the oral phase comes under voluntary control with nasal breathing and an open airway, allowing children to handle various food textures. The triggering of the swallowing reflex is a basic reflex, which provides safe and efficient swallowing, preventing aspiration and retention of food in the pharyngeal stage. The swallow reflex is an involuntary activity, even if it can be controlled voluntarily. The pharyngeal phase of swallowing begins with the initiation of the pharyngeal swallow response as the food/ fluid bolus passes through the pharynx and is under reflexive neurological control. The oesophageal phase of swallowing begins with the opening of the upper oesophageal sphincter (UES) and ends as the food/ fluid bolus passes through the lower oesophageal sphincter into the stomach and constitutes an involuntary activity. Table 1 demonstrates the typical swallow sequence of children during the oral, pharyngeal and oesophageal phases [10].

Additionally to the complexity of the swallowing process, children's feeding and swallowing skills are affected by maturational changes. The pharyngeal swallow becomes obvious between 10- and 11-weeks' gestation and sucking between 18- and 24-weeks' gestation. However, it is evident that sucking and swallow-

Table 1. *Typical swallow sequence of children during the swallowing phases.*

Phases of Swallowing	Typical swallow sequence
Oral phase	• Preparing the bolus for swallow • Chewing (if necessary) • Transferring the bolus from the oral cavity into the pharynx
Pharyngeal phase	• Velopharyngeal closure occurs to close off the nasopharynx • Posterior movement of the base of tongue • Pharyngeal closure through contraction of the superior, middle, and inferior pharyngeal constrictors • Adduction of the vocal folds with brief cessation of respiration • Hyolaryngeal excursion and closure of the larynx through epiglottic tilt • Opening of the upper oesophageal sphincter through relaxation of the cricopharyngeus muscle and biomechanical forces contributed through hyolaryngeal excursion
Oesophageal phase	• Peristaltic action of the oesophagus • Moving the bolus into the stomach.

ing coordination matures between 33- and 36-weeks' gestation [12]. The neurologically intact full-term infant is born with a number of oropharyngeal reflexes, which support the feeding and swallowing procedure. These include the rooting reflex, the gag reflex, the phasic bite, the transverse tongue reflex, the tongue protrusion, suckling and swallow reflex [13]. At the age of 4-6 months, infants come to a period of transitional feeding until approximately 3 years of age. During this period, neurodevelopmental processes and anatomical and structural changes lead to the inhibition of basic feeding reflexes and support the development of more refined oral sensorimotor and swallowing skills. This progress in feeding and swallowing procedure enables the ingestion of various complex food and fluid textures, from various feeding utensils, and continues until approximately 3 years of age [14]. In a neurologically intact infant with a normal airway, cough may be an indicator of aspiration. While cough can be elicited at birth, it is a reflex that develops over time, providing airway protection [15].

PAEDIATRIC FEEDING AND SWALLOWING DISORDERS

The term dysphagia is defined as a feeding and swallowing disorder, which includes dysfunction of the motor or sensory aspects of swallowing [16], and originates from impairment in the mouth (including mandible, lips, teeth, tongue, cheeks, hard and soft palates), the pharynx, the larynx, and the upper oesophagus [12]. Paediatric dysphagia may include impairment to the cranial nerves: trigeminal (V), facial (VII), glossopharyngeal (IX), vagus (X), accessory (XI), and hypoglossal (XII) [17]. Functionally, paediatric dysphagia may include difficulties with sucking, drinking, chewing, swallowing, eating, progressing from breast or bottle to pureed or solid foods, control of saliva, ingesting medication, airway protection, while children may demonstrate gagging, choking, or vomiting incidents with the introduction of pureed or solid foods [18]. Dysphagia is a symptom [19] that involves the oral, pharyngeal or oesophageal phase of swallowing and can negatively impact health and quality of life (QoL) [20]. Paediatric dysphagia may have subsequent effects on everyday activity and participation [21].

For some children, the presence of a dysphagic symptom may signal the occurrence of a disease [22]. Children with dysphagia can present with various feeding and swallowing disorders affecting any or all of the swallowing phases. The most common paediatric dysphagia symptoms relevant to the oral phase are absent oral reflexes or primitive/neurological oral reflexes, inadequate lip closure, weak or uncoordinated suck, immature or disordered biting and/or chewing, poor bolus propulsion and poor bolus containment, impaired clearance of the bolus from the mouth following the swallow, oral sensorimotor impairments [1, 10, 23, 24]. The triggering of the swallow reflex may be affected when an infant or a child demonstrates absent swallow reflex, delayed triggering of the swallow and suck/swallow/breath incoordination [10]. Dysphagic symptoms related to the pharyngeal phase include delayed pharyngeal swallow response/ initiation, laryngeal penetration, oropharyngeal aspiration and silent aspiration, choking, pharyngeal residue, nasopharyngeal reflux [1, 10, 23]. Symptoms of dysphagia related to the oesophageal phase are residue above the upper oesophageal sphincter and residue or pooling in the oesophagus because of motility problems or gastroesophageal reflux [1].

CONDITIONS ASSOCIATED WITH PAEDIATRIC FEEDING AND SWALLOWING DISORDERS

It is increasingly recognised that feeding and swallowing disorders in the paediatric population are multifactorial. The domains that underlie paediatric feeding and swallowing disorders are medical, nutritional, feeding skills, and psychosocial [25].

The most common medical issues that a child with feeding and swallowing disorders may deal with are gastroesophageal reflux, neurological and cardiopulmonary conditions [24, 18, 25, 26], craniofacial anomalies [18, 24, 26], renal disease [24, 26], food allergies/ intolerance [18, 24], neurodevelopmental disorders (Autism Spectrum Disorders) [25], genetic, oncologic and metabolic causes [26]. Table 2 depicts common medical diagnoses associated with paediatric feeding and swallowing disorders [5, 27, 28].

Children with feeding and swallowing disorders may consume a limited quality, quantity, and/or variety of foods. As a result, they may deal with nutritional issues which include malnutrition, overnutrition, micronutrient deficiency or toxicity, and dehydration [25].

Feeding skill factors include impairment in oral sensory functioning, oral motor functioning, pharyngeal sensation functioning and pharyngeal motor functioning [25]. Disorders in gross-motor development, such as reduced postural stability or head control, and sensory processing issues, such as regulation disorders, sensory discrimination, and sensory-based motor delays are factors that lead to feeding and swallowing disorders

Table 2. *Common medical conditions associated with Paediatric Feeding and Swallowing Disorders.*

Neurological/ Neurodevelopmental	Prematurity, Cerebral Palsy, Perinatal Asphyxia, Acquired Brain Injury, Traumatic Brain Injury, Hypoxic Brain Injury, Cranial Nerve Injury, Myopathy, Congenital Myopathy, Myasthenia Gravis, Myotonic Dystrophy, Muscular Dystrophy, Seizures/ Seizure Disorders, Laryngeal Paralysis, Vocal Cord Paralysis, Autism Spectrum Disorder (ASD)
Structural	Cleft Lip/Palate, Craniofacial Anomalies, Macroglossia, Laryngomalacia, Laryngotracheal Cleft, Laryngeal Stenosis/ Cleft, Oesophageal Atresia, Tracheomalacia, Tracheosophageal Fistula, Iatrogenic (Tracheostomy)
Cardiopulmonary	Aspiration Lung Disease, Bronchopulmonary Dysplasia, Congenital Heart Disease, Respiratory Syncytial Virus
Gastrointestinal	Food Allergy/ Intolerance, Gastroesophageal Reflux Disease, Necrotizing Enterocolitis, Oesophageal Dysmotility
Genetic	Down Syndrome, Pierre-Robin Sequence, Prader-Willi, CHARGE Syndrome, Treacher-Collins Syndrome, Cornelia de Lange Syndrome

in the paediatric population [18].

Children with oral sensory functioning issues may demonstrate hyposensitivity, as they do not feel the food in their mouth and as a result they illustrate limited bolus formation, loss of food from the mouth, and gagging or refusal of liquids and food textures that provide inadequate sensory input [25]. Children with hypersensitivity may demonstrate gagging with specific food type/ texture/ size/ flavour/ temperature/ viscosity/ appearance [29, 30], excessive chewing, and limited variety of intake [25]. Sensory regulation problems can cause feeding and swallowing difficulties, such as food refusal and self-limited diets [31]. Children with oral motor functioning issues may reveal inefficient intake, messy eating, restricted control of liquids and food, slow or ineffective bolus formation and propulsion, gagging during bolus formation, and post swallowing residues [32].

Goday et al. cite the complications which are related to impairment in pharyngeal sensation and motor functioning [25]. Impairment in pharyngeal sensation functioning includes poor awareness of bolus location, delayed pharyngeal swallow response, nasopharyngeal reflux, post-swallow residue, laryngeal penetration and oropharyngeal aspiration. Impairment in pharyngeal motor functioning inhibits pharyngeal movements, as reduction in the strength and coordination of pharyngeal constrictors, velar and laryngeal elevation, and vocal fold closure may be noticed. When a child's feeding skills are not safe, age appropriate and efficient, feeding and swallowing disorder is hard to avoid.

Psychosocial factors in the child and/or caregiver can contribute to feeding and swallowing disorder [33], given that feeding occurs in the context of the caregiver-child dyad. Psychosocial factors are characterized as developmental factors, mental and behavioral health problems, social factors, or environmental factors [34, 35]. Paediatric feeding and swallowing disorders may become obvious with various behaviors. These behaviors include child's learned aversions to food, disruptive behavior, food overselectivity, failure to advance to age-appropriate diet despite adequate skills, grazing, child's and/or caregiver's stress or inappropriate caregiver's strategies to improve child's nutritional status [34].

THE ROLE OF THE SLP AND THE MULTIDISCIPLINARY TEAM IN PAEDIATRIC FEEDING AND SWALLOWING DISORDERS

Given that paediatric feeding and swallowing disorders occur in conjunction with multiple factors, a multidisciplinary approach is critical for the evaluation and appropriate treatment of these difficulties [26, 27]. Depending on the age and severity of feeding and swallowing disorders, children may receive intervention in various settings [36]. A multidisciplinary team consists of SLPs, occupational therapists (OT), physiotherapists (PT), nurses, dieticians, physicians, paediatricians, gastroenterologists and radiologists when radiological studies are necessary [28, 37]. Definitely, all professionals are not necessary for all children, as they may have different issues, which change over time [1]. SLPs are recognised by the American Speech-Language-Hearing Association (ASHA) as the primary experts involved, and they are responsible for the evaluation and management of dysphagia [23]. Specifically, the professional roles and responsibilities of SLPs involve the conduction of

a comprehensive assessment, including clinical and instrumental evaluations, the participation in decision-making regarding the appropriateness of instrumental evaluation procedures and follow-up, the diagnosing of feeding and swallowing disorders and the recognition of behavioural feeding problems, the education of parents/ caregivers to prevent complications related to feeding and swallowing disorders and the collaboration with other professionals, family members, caregivers in order to facilitate the treatment plan.

FEEDING AND SWALLOWING EVALUATION PROCESS

Commonly, physicians refer children to an SLP as seemingly deal with behavioural issues. A child may reveal refusal to eat, a limited diet based upon texture, taste and visual appearance, or difficult transition from breast or bottle to pureed or solid foods, accompanied by gagging, choking, or vomiting incidents with the introduction of food [18]. An in-depth SLP evaluation may reveal that all these difficulties are related with medical and developmental issues. Consequently, behavioural issues may develop secondary to feeding and swallowing difficulties in the oral phase.

Clinical Assessment Based on the World Health Organisation Concepts

According to the ASHA, the assessment of feeding and swallowing disorders should follow the International Classification of Functioning, Disability and Health (ICF) framework [38]. The World Health Organization's ICF, provides a standard language for international and multidisciplinary use, and incorporates (i) body function and structures, (ii) activities and participation, (iii) environmental factors and (iv) personal factors [39]. Studies provide a description of the different components of the ICF in relation to feeding and swallowing disorders [1, 40]. Specifically, the body functions components involve the swallowing procedure including specific movements (i.e. sucking, biting, chewing). The body structures components relate to the neurological system and the structures needed to carry out the physical act of the feeding procedure (i.e. teeth, tongue, jaw, larynx). Impairment of body function and structures, is relevant to deficits, including motor and sensory skills, neuromuscular and orthopaedic conditions or respiratory status. Activities involve the child's activity restrictions during mealtime, including self-feeding skills or adaptive equipment needs. Participation is linked to the social and physical mealtime settings where the child participates, including home and school. Environmental factors include facilitators or barriers, such as the availability of appropriate food texture or the support of family members. Personal factors include demographic characteristics (i.e. age, race), personality traits and preferences. For a comprehensive and in-depth assessment, information related to ICF components is necessary, while participation is the primary consideration in a feeding and swallowing assessment [1].

Screening Questions for Primary Care Physicians

SLPs are the most suitable professionals who can carry out an effective feeding and swallowing assessment. However, because of the fact that physicians are often the first professionals who meet children and their family, they should be able to elicit information regarding child's feeding and swallowing skills from parents/ caregivers, so as to refer them to an SLP, if indicated. Arvedson et al, proposes a list of questions which physicians may find helpful to determine if a child faces feeding and swallowing difficulties [1]:

- "How long does it take to feed the child?"
- "Is the child totally dependent on others for feeding? Does the child do some assisted feeding or some independent feeding?"
- "Does the child refuse food?"
- "Are mealtimes stressful?"
- "Has the child slowed or stopped gaining weight in the previous 2-3 months?"
- "Are there any signs of respiratory distress?"
- "Does the child vomit regularly? When? Under what circumstances does the vomiting occur? Can parents estimate the volume per event?"
- "Does the child get irritable or become lethargic during mealtimes?"

There are two methods for diagnosing paediatric dysphagia: The clinical feeding and swallowing evaluation and the instrumental evaluation of swallowing. It is essential for children to have a clinical feeding and swallowing evaluation prior to the instrumental evaluation of swallowing to enable diagnostic planning and priorities of the procedure.

Clinical Feeding and Swallowing Evaluation

The clinical feeding and swallowing evaluation includes a review of child's medical status, gross, fine and oral-motor development, sensory processing and nutritional status [8]. According to Arvedson et al, the clinical feeding and swallowing evaluation includes

history taking, pre-feeding evaluation, oral structure and function assessment and examination of cranial nerves function [1], as is described below:

Review of family, medical, development and feeding history, obtained from medical charts, clinicians and teachers, parents/ caregivers. The history should provide information on feeding environment, parent/ caregiver-child interaction and parental worries [1, 4, 41, 42].

The pre-feeding evaluation includes observation of saliva control, general posture, positioning and movement patterns, respiratory patterns, the child's response to stimulation (tactile, visual, auditory, olfactory), the level of responsiveness and mental state, the level of interaction and communication, self-regulation and self-calming [1, 43].

The oral structure and function assessment are carried out, evaluating structure, symmetry, tone and movement of the facial features including the jaw, lips, cheeks, tongue, hard and soft palate and uvula.

Feeding and swallowing evaluation includes the examination of cranial nerves function by evaluating oral reflexes, laryngeal function and movement during feeding related tasks [1].

A complete assessment process should include evaluation of feeding readiness, breast and bottle feeding (where age appropriate), drinking from various utensils, spoon feeding and eating solids. Initial interventions (trialing various food and fluid textures, taste, temperature) may be trialled to assess their effects, providing essential information on treatment planning [1].

The high incidence of children with feeding and swallowing disorders because of various reasons, results in a great need of validated non-instrumental swallowing and feeding assessment in paediatric population. The Schedule for Oral-Motor Assessment (SOMA) [44-46] and the Dysphagia Disorder Survey (DDS) [47] are two of the more commonly used standardized clinical measures of oral motor and feeding assessment for identification of feeding and swallowing disorders in paediatric populations [47, 48].

Instrumental Evaluation of Swallowing

Instrumental evaluation of swallowing is conducted to understand the nature and pathophysiology of the swallowing dysfunction and to obtain the information needed to develop the most appropriate treatment plan.

The two most used instrumental evaluations of swallowing for the paediatric population are the Videofluoroscopic Swallowing Study (VFSS) and the Flexible Endoscopic Evaluation of Swallowing (FEES). The decision regarding which instrumental evaluation of swallowing is necessary depends on the functional and anatomical process to be evaluated [49].

Videofluoroscopic Swallowing Study (VFSS)

The VFSS constitutes an instrumental evaluation which is performed by an SLP and a radiologist [50] and provides dynamic imaging of the phases of swallowing: the oral phase, the pharyngeal phase and the upper oesophageal phase of swallowing. Depending on which structures is essential to be evaluated, the VFSS can be carried out in the anterior-posterior or lateral view (more common) [1]. During the VFSS, the position of the child is similar to their usual feeding position (upright in a suitable paediatric chair, semi-reclined in an infant seat, or side-lying for some infants) [50]. The SLP is responsible for the preparation and presentation of various amounts and consistencies of barium sulfate, with or without food [51]. Food and fluids may be given to the children in various textures (such as purees, lumpy, mashed (semisolid) textures, chewable solid, thickened fluids (mild, moderate, thick) and thin fluid [23, 50] and are given with their regular utensils (bottles, teats, cups, spoons, etc) [50]. During this radiographic examination, variations, such as postural variations, altering head and neck position, changing food/ fluid bolus size and/ or texture, effect of additional dry swallows, food/ fluid bolus placement in the mouth, changing utensils and therapeutic manoeuvres, may be assessed to determine their effect [23] and also to facilitate the treatment plan. The VFSS can identify delays in the pharyngeal transit time, dysfunction in the velopharyngeal closure and laryngeal penetration and aspiration, before or during the swallow [41].

Flexible Endoscopic Examination of Swallowing (FEES/ FEES-ST)

The FEES is an instrumental evaluation which is completed by an SLP and a paediatric Otolaryngologist (ENT) [1, 52] and provides visualisation of events, which occur before and after the pharyngeal swallow [1]. A flexible endoscope is inserted through the child's nose, which allows for the visualisation of nasal, pharyngeal, and laryngeal structures. FEES is possible to be performed at the bedside and the child should swallow a bolus of food and/ or fluid that includes an amount of methylene blue. Food and fluids can be given to the children in various textures and with multiple utensils, and compensatory and therapeutic swallowing tech-

niques can be implemented [53]. During this examination, the examiner(s) may wish to perform a sensory test (FEESST), which uses air pressure to evaluate laryngeal reflexes [1]. The swallowing function parameters which are assessed during FEES/ FEESST involve pharyngeal pooling of secretions, premature spillage into pharynx, laryngeal penetration, aspiration, vocal fold paresis, gag reflex and laryngeal adductor reflex (LAR) [49].

Other Diagnostic Evaluations

While VFSS and FEES exams are the most commonly instrumental feeding and swallowing assessments in paediatric practice, other methods have received attention for their diagnostic usefulness in paediatric populations. Ultrasonography (US) is carried out in order to visualize temporal relationships between movement patterns during the oral and pharyngeal phase in infants and young children [1]. Specialized diagnostic evaluations may be essential for children who deal with respiratory issues. These evaluations may include chest radiographs, pulmonary function tests, high-resolution CT, and bronchoscopy. Endoscopy may be crucial for children who deal with gastrointestinal issues [27].

MANAGEMENT OF PAEDIATRIC FEEDING AND SWALLOWING DISORDERS

The multidisciplinary approach plays a leading role in the diagnosis and management of dysphagia in the paediatric population [27]. In order the most appropriate dysphagia intervention to be achieved, SLPs should focus on improved individual functioning across all ICF components.

Common feeding and swallowing treatment strategies that address the pathophysiology of swallowing dysfunction in infants and children include medical or surgical intervention, Oral-Motor Exercises (OME), adapted feeding equipment, diet modifications, compensatory strategies, Neuromuscular Electrical Stimulation (NMES), supplemental feeding, behaviourally based intervention, environmental changes, and also oral hygiene.

For children with physical or anatomic issues (laryngeal cleft or tracheoesophageal fistula), inflammatory issues (eg, esophagitis), gastroesophageal reflux disease (GERD) [27] and congenital abnormalities preventing optimal swallow function (stricture, severe reflux, cleft palate, etc), medical or surgical interventions can be used to treat dysphagia [43]. In very severe cases of chronic aspiration, laryngotracheal separation may be considered by the medical team [54]. For children with tracheotomies, a cap or a speaking valve can be used to increase pressure, aiding in the development and function of a more normal oropharynx [43].

SLPs working with children who deal with feeding and swallowing disorders frequently incorporate OME into their treatment plan. OME constitute (i) active exercises, (ii) passive exercises, and (iii) sensory applications [55]. Active exercises include active range of motion, stretching, and strength training and are used to increase strength, endurance, and power of the face, tongue, lips, and palate muscles [56]. Passive exercises include massage, tapping, stimulation, stroking, vibration, and passive range of motion exercises, provided by the SLP with little action from the child [36]. Passive exercises aim to provide sensory input, improve circulation, enhance joint flexibility, reduce abnormal oral reflexes, facilitate normal muscle tone, and desensitize the oral cavity [57]. Sensory applications constitute the use of heat, cold, electrical stimulation, high-frequency vibration, or other agents to muscle tissues. Sensory applications may be applied to enhance sensory awareness to initiate a swallow response or to strengthen the swallowing musculature [36]. Although OME are widely applied by SLPs, the use of them outside of a functional setting remains controversial. Both Beckman et al, and Rosenfeld-Johnson et al, support that the application of OME outside of a functional setting can improve the swallowing process [58, 59] . However, other authors support that there is insufficient evidence to determine the effects of OME on paediatric populations with feeding and swallowing disorders without the use of functional therapy tasks that directly impact on eating and drinking skills and/or safety [36, 60, 61].

When the child has improved sensory regulation and their oral-motor skills, a therapeutic feeding program can be designed, including adaptive utensils, various bottles, nipples, cups, spoons and therapeutic straws [62]. The adapted feeding equipment is used to control liquid flow and bolus size and shape. Diet modifications can improve the timing, coordination and sensory input of food and liquid. The modification of food and liquids is achieved by modifying viscosity, texture, temperature or taste [63]. Compensatory swallowing manoeuvers can lead to improved oropharyngeal musculature, airway protection and safe transition of food/ liquid bolus, by adjusting the child's position during the feeding process [63]. These manoeuvers may be appropriate for older children with signs of aspiration, depending on the pathophysiology and the ability of children to follow

directions [27]. Treatment recommendations, relevant to the most appropriate compensatory swallowing manoeuver, are made based on instrumental examination findings. NMES is usually used as a swallowing therapy in case of pharyngeal dysphagia. However, there are studies which support that the impact of NMES on dysphagia management is controversial. According to a study, the use of NMES of anterior neck muscles in a heterogeneous group of children with swallowing dysfunction did not impact on swallow function compared with the control group, which received only OME and diet modifications [64]. On the other hand, NMES constitutes an effective intervention for different paediatric caseloads with pharyngeal phase dysfunction as it is evidenced by a case series design [65].

In case of severe dysphagia, recommendation of alternative avenues of nutrition and hydration (including nasogastric tube or gastrostomy) may be made, so that safe swallowing and adequate nutrition/ hydration to be ensured [43]. Despite the feeding tube placement, SLPs and caregivers should make efforts to offer tastes to children for swallowing practice [27].

For children with behavioral feeding issues, behavioural approaches focus on parent/ caregiver education, behavioral modifications and the presentation of shaping, prompting, modelling, stimulus fading and antecedent manipulation [63]. Behaviourally based interventions may have beneficial impact on feeding behaviour [66]. Environmental changes, such as alteration of the temperature, light and noise in the child's feeding environment, may relax infants and young children [67], demonstrating improved feeding skills. Oral hygiene is an intimate component of all treatment techniques because poor oral hygiene is a risk factor for lung infections.

Overall, the specific treatment strategies, used by SLPs in collaboration with the multidisciplinary team, are individualized and depend on the nature of the feeding and swallowing disorder, as well as the child's response to treatment interventions.

CONCLUSION

The development of feeding and swallowing skills begins in the fetal period and continues until early childhood. Feeding and swallowing dysfunction in the paediatric population may be attributed to various aetiologies and may be manifested with different signs and symptoms. The primary expert for the evaluation and management of paediatric dysphagia is the SLP. However, the collaboration between SLPs and multidisciplinary team plays a critical role in decision making, as dysphagia is multifactorial. Feeding and swallowing evaluation in the paediatric population involves clinical and instrumental swallowing evaluation and provides vital information to diagnosis and treatment planning. The primary goal of all therapeutic approaches is the safeguarding of adequate nutrition, hydration, and airway protection.

Conflict of interest disclosure: *None to declare*

Declaration of funding sources: *None to declare.*

Author contributions: *Angeliki Zarkada was responsible for literature search and analysis and collection of data, interpreting the data, drafting the manuscript; Rigas Dimakopoulos was responsible for the revision of the manuscript for important intellectual content; Terpsithea Germani was responsible for literature search; Angeliki Mantzari was responsible for literature search; Georgia Karadimou was responsible for literature search. All authors approved the submitted version of the manuscript.*

REFERENCES

1. Arvedson JC. Assessment of pediatric dysphagia and feeding disorders: clinical and instrumental approaches. Dev Disabil Res Rev. 2008;14(2):118-27.
2. Equit M, Pälmke M, Becker N, Moritz AM, Becker S, von Gontard A. Eating problems in young children–a population-based study. Acta Paediatr. 2013;102(2):149-55.
3. McDermott BM, Mamun AA, Najman JM, Williams GM, O'Callaghan MJ, Bor W. Preschool children perceived by mothers as irregular eaters: physical and psychosocial predictors from a birth cohort study. J Dev Behav Pediatr. 2008;29(3):197-205.
4. Tutor JD, Gosa MM. Dysphagia and aspiration in children. Pediatr Pulmonol. 2012;47(4):321-37.
5. Miller CK. Updates on pediatric feeding and swallowing problems. Curr Opin Otolaryngol Head Neck Surg. 2009;17(3):194-9.
6. Cooper-Brown L, Copeland S, Dailey S, Downey D, Petersen MC, Stimson C, et al. Feeding and swallowing dysfunction in genetic syndromes. Dev Disabil Res Rev. 2008;14(2):147-57.
7. Durvasula VS, O'Neill AC, Richter GT. Oropharyngeal dysphagia in children: mechanism, source, and management. Otolaryng Clin N Am. 2014;47(5):691-720.
8. Arvedson JC, Brodsky L, Lefton-Greif MA. Pediatric swallowing and feeding: Assessment and management: Plural Publishing; 2019.
9. Arvedson J, Brodsky L, Reigstad D. Clinical feeding and swallowing assessment. Pediatric swallowing and feeding 2nd edn Canada: Thomson Delmar Learning. 2002:283-340.

10. Dodrill P, Gosa MM. Pediatric dysphagia: physiology, assessment, and management. Ann Nutr Metab. 2015;66(Suppl. 5):24-31.
11. Matsuo K, Palmer JB. Anatomy and physiology of feeding and swallowing: normal and abnormal. Phys Med Rehabil Clin N Am. 2008;19(4):691-707.
12. Arvedson J, Brodsky L. Pediatric swallowing and feeding assesment and development. San Diego (CA): Singular. 1993.
13. Hall KD. Pediatric dysphagia resource guide: Singular; 2001.
14. Kramer SS, Eicher PM. The evaluation of pediatric feeding abnormalities. Dysphagia. 1993;8(3):215-24.
15. Chang AB. The physiology of cough. Paediatr Respir Rev. 2006;7(1):2-8.
16. Lamm NC, De Felice A, Cargan A. Effect of tactile stimulation on lingual motor function in pediatric lingual dysphagia. Dysphagia. 2005;20(4):311-24.
17. Cichero JA, Murdoch BE. Dysphagia: foundation, theory and practice: JWS; 2006.
18. Overland L. A sensory-motor approach to feeding. Perspectives on Swallowing and Swallowing Disorders (Dysphagia). 2011;20(3):60-4.
19. Piemonte M. Fisiopatologia della deglutizione. Atti XIV Giornata Italiana di Otoneurologia, Senigallia. 1997.
20. Farneti D, Consolmagno P. The Swallowing Centre: rationale for a multidisciplinary management. Acta Otorhinolaryngo. 2007;27(4):200.
21. World Health Organization (WHO). International classification of functioning. Disability and Health (ICF). 2001:153-5.
22. Group YICSS. Clinical signs that predict severe illness in children under age 2 months: a multicentre study. The Lancet. 2008;371(9607):135-42.
23. American Speech-Language-Hearing Association (ASHA). Paediaric Dysphagia. 2015.
24. Field D, Garland M, Williams K. Correlates of specific childhood feeding problems. J Paediatr Child Health. 2003;39(4):299-304.
25. Goday PS, Huh SY, Silverman A, Lukens CT, Dodrill P, Cohen SS, et al. Pediatric feeding disorder: consensus definition and conceptual framework. J Pediatr Gastroenterol Nutr. 2019;68(1):124.
26. Rommel N, De Meyer A-M, Feenstra L, Veereman-Wauters G. The complexity of feeding problems in 700 infants and young children presenting to a tertiary care institution. J Pediatr Gastroenterol Nutr. 2003;37(1):75-84.
27. Lefton-Greif MA. Pediatric dysphagia. Phys Med Rehabil Clin N Am. 2008;19(4):837-51.
28. Miller CK. Aspiration and swallowing dysfunction in pediatric patients. Infant Child Adolesc Nutr . 2011;3(6):336-43.
29. Farrow CV, Coulthard H. Relationships between sensory sensitivity, anxiety and selective eating in children. Appetite. 2012;58(3):842-6.
30. Naish KR, Harris G. Food intake is influenced by sensory sensitivity. PLoS One. 2012;7(8).
31. Twachtman-Reilly J, Amaral SC, Zebrowski PP. Addressing feeding disorders in children on the autism spectrum in school-based settings: Physiological and behavioral issues. Lang Speech Hear Ser. 2008.
32. Weir K, McMahon S, Barry L, Ware R, Masters IB, Chang AB. Oropharyngeal aspiration and pneumonia in children. Pediatr Pulmonol. 2007;42(11):1024-31.
33. Davies W, Satter E, Berlin KS, Sato AF, Silverman AH, Fischer EA, et al. Reconceptualizing feeding and feeding disorders in interpersonal context: the case for a relational disorder. J Fam Psychol. 2006;20(3):409.
34. Burklow KA, Phelps AN, Schultz JR, McConnell K, Rudolph C. Classifying complex pediatric feeding disorders. J Pediatr Gastroenterol Nutr. 1998;27(2):143-7.
35. Berlin KS, Lobato DJ, Pinkos B, Cerezo CS, LeLeiko NS. Patterns of medical and developmental comorbidities among children presenting with feeding problems: a latent class analysis. J Dev Behav Pediatr. 2011;32(1):41-7.
36. Arvedson J, Clark H, Lazarus C, Schooling T, Frymark T. The effects of oral-motor exercises on swallowing in children: an evidence-based systematic review. Dev Med Child Neurol. 2010;52(11):1000-13.
37. Cowpe E, Hanson B, Smith CH. What do parents of children with dysphagia think about their MDT? A qualitative study. BMJ open. 2014;4(10):e005934.
38. American Speech-Language-Hearing Association (ASHA). Preferred practice patterns for the profession of speech-language pathology. 2004.
39. World Health Organization (WHO). International Classification of Functioning, Disability, and Health: Children & Youth Version: ICF-CY: World Health Organization; 2007.
40. Threats TT, editor Use of the ICF in dysphagia management. Seminars in speech and language; 2007: Thieme Medical Publishers.
41. Wallis C, Ryan M. Assessing the role of aspiration in pediatric lung disease. Pediat Aller Imm Pul. 2012;25(3):132-42.
42. de Benedictis FM, Carnielli VP, de Benedictis D. Aspiration lung disease. Pediatr Clin N Am. 2009;56(1):173-90.
43. Prasse JE, Kikano GE. An overview of pediatric dysphagia. Clin Pediatr. 2009;48(3):247-51.
44. Reilly S, Skuse D, Mathisen B, Wolke D. The objective rating of oral-motor functions during feeding. Dysphagia. 1995;10(3):177-91.
45. Reilly S, Skuse D, Wolke D. Schedule for Oral Motor Assessment (SOMA). 2000.
46. Skuse D, Stevenson J, Reilly S, Mathisen B. Schedule for oral-motor assessment (SOMA): methods of validation. Dysphagia. 1995;10(3):192-202.
47. Sheppard JJ, Hochman R, Baer C. The dysphagia disorder survey: validation of an assessment for swallowing and feeding function in developmental disability. Dev Disabil Res Rev. 2014;35(5):929-42.
48. Ko MJ, Kang MJ, Ko KJ, Ki YO, Chang HJ, Kwon J-Y. Clinical usefulness of Schedule for Oral-Motor Assessment (SOMA) in children with dysphagia. Ann Rehabil Med. 2011;35(4):477.
49. Arvedson JC. Swallowing and feeding in infants and young children. GI Motility online. 2006.
50. Hiorns MP, Ryan MM. Current practice in paediatric vide-

ofluoroscopy. Pediatr Radiol. 2006;36(9):911-9.
51. Crary MA, Groher ME. Introduction to adult swallowing disorders: Butterworth-Heinemann Medical; 2003.
52. Allison J. Instrumental Evaluation of Pediatric Dysphagia: FEES Versus Videofluoroscopy. Research Papers. 2011; Paper 42.
53. Hiss SG, Postma GN. Fiberoptic endoscopic evaluation of swallowing. The Laryngoscope. 2003;113(8):1386-93.
54. Cook SP. Candidate's Thesis: Laryngotracheal separation in neurologically impaired children: Long-term results. The Laryngoscope. 2009;119(2):390-5.
55. Clark HM. Neuromuscular treatments for speech and swallowing. Am J Speech Lang Pathol. 2003.
56. Burkhead LM, Sapienza CM, Rosenbek JC. Strength-training exercise in dysphagia rehabilitation: principles, procedures, and directions for future research. Dysphagia. 2007;22(3):251-65.
57. Ottenbacher K, Scoggins A, Wayland J. The effectiveness of a program of oral sensory-motor therapy with the severely and profoundly developmentally disabled. OTJR. 1981;1(2):147-60.
58. Beckman D. Beckman oral motor interventions. Course pack accompanying Oral Motor Assessment and Intervention workshop, Charlotte, NC; 2001.
59. Johnson SR, S R-J. Oral-Motor exercises for speech clarity. Talk Tools, Tucson, Arizona. 1999.
60. Harding C, Cockerill H. Managing eating and drinking difficulties (dysphagia) with children who have learning disabilities: What is effective?. Clin Child Psychol P. 2015;20(3):395-405.
61. Martin RE, Goodyear BG, Gati JS, Menon RS. Cerebral cortical representation of automatic and volitional swallowing in humans. J Neurophysiol. 2001;85(2):938-50.
62. Rosenfeld-Johnson S. Oral placement therapy for speech clarity and feeding: Talk Tools Therapy; 2009.
63. Piano B. Indirect approaches: A systematic review of paediatric dysphagia interventions. 2015.
64. Christiaanse ME, Mabe B, Russell G, Simeone TL, Fortunato J, Rubin B. Neuromuscular electrical stimulation is no more effective than usual care for the treatment of primary dysphagia in children. Pediatr Pulmonol. 2011;46(6):559-65.
65. Rice KL. Neuromuscular electrical stimulation in the early intervention population: A series of five case studies. IJAHSP. 2012;10(3):9.
66. Sharp WG, Jaquess DL, Morton JF, Herzinger CV. Pediatric feeding disorders: A quantitative synthesis of treatment outcomes. Clin Child Fam Psychol Rev. 2010;13(4):348-65.
67. Morgan AT, Dodrill P, Ward EC. Interventions for oropharyngeal dysphagia in children with neurological impairment. Cochrane Database Syst Rev. 2012(10).

Corresponding author:

Angeliki Zarkada,
16 Kononos street, 11634 Athens,
Tel.: +30 6976 731819
E-mail: aggelikazark@hotmail.gr

Acknowledgments

We would like to thank Dr. Katerina Karaivazoglou for English-language editing and Dr. Ioanna Aggeletopoulou for scientific editing of the manuscripts.

The journal "Achaiki Iatriki" publishes original papers on clinical and basic research from all areas of the health sciences including healthcare. The journal is published exclusively in English. Manuscripts should conform to the guidelines set out in "Uniform Requirements for Manuscripts Submitted to Biomedical Journals" by the International Committee of Medical Journal Editors (http:/www.icmje.org).

COVER LETTER

A submission letter to the Editor should accompany the manuscript and contain the following:

- The manuscript has not been published previously, and is not under consideration for publication elsewhere.
- Acknowledgment of grants or financial support.
- The manuscript has been approved by all authors.

INFORMATION ABOUT ARTICLE TYPES

The Editors will consider and publish the following:

1. Original research articles
2. Narrative Reviews
3. Systematic Reviews and Meta-analyses
4. Editorials
5. Letters to the Editor
6. Case Reports

Original research articles

The maximum length of the main text is 3,500 words excluding the abstract, references, tables, and figure legends. A maximum of 6 tables and/or figures is allowed. References should not exceed a maximum of 100.

Narrative Reviews / Systematic Reviews / Meta-analyses

These manuscripts are solicited and unsolicited manuscripts that feature an organized and detailed review of the scientific literature about a particular topic. This section is peer-reviewed and acceptance for publication is not guaranteed. The maximum length of the main text is 5,000 words excluding the abstract, references, tables, and figure legends. A maximum of 6 tables and/or figures to summarize critical points is highly desirable. References should not exceed a maximum of 150.

Editorials

Editorials are usually solicited by the Editor. The maximum length is 1500 words excluding the references, tables, and figure legends. One table or 1 figure is allowed. References should not exceed a maximum of 20. Editorials may have a maximum of three (3) authors.

Letters to the Editor

Letters to the Editor will be considered for publication if they are related to articles published in recent issues of the Achaiki Iatriki Journal. The maximum length is 800 words (excluding references, table, and figure legend). A total number of 1 table or figure is allowed and up to 10 references. Such letters will be passed to the authors of the original paper, who will be offered an opportunity to reply. Letters to the Editor may have a maximum of two (2) authors.

Case Reports

Case reports should ideally include a short introduction, the case presentation and a brief discussion. The maximum length is 1500 words (excluding references, tables, and figure legend). A total number of 2 tables or figures is allowed. References should not exceed a maximum of 15.

Formatting guide

The articles must by typewritten and double spaced. They should include the following sections, each starting on a separate page:

- Title Page
- Abstract and Key Words
- Main Text
- Acknowledgements
- References
- Tables
- Figures

Margins should be not less than 2.5 cm. Pages should be numbered consecutively.

Abbreviations

Do not use non-standard abbreviations. The use of abbreviations in the title and abstract should be avoided. Abbreviations should be defined on their first appearance in the text; those not accepted by international bodies should be avoided.

Title page

The title page should include:

- Title of the manuscript
- Short title which will be used as a running head
- Full name of each author
- Full location of department and institution where work was performed
- Name and address for correspondence, including fax number, telephone number, and e-mail address.
- Conflict of interest disclosure.
- Declaration of funding sources.
- Author Contributions according to the following criteria for authorship: conception and design; analysis and interpretation of the data; drafting of the article; critical revision of the article for important intellectual content; final approval of the article.

Abstract

For Original Articles, structured abstracts should be 250 words or less and include the following sections: Background, Methods, Results and Conclusion. Review articles should carry an unstructured abstract which should not exceed 200 words.

Key words

The abstract should be followed by a list of 3–5 keywords which will assist the cross-indexing of the article and which may be published separated by semicolons.

Main Text

For the main body of the text, the recommended structure of

the manuscript is:
- Introduction
- Materials and Methods
- Results
- Discussion

Define abbreviations at first mention in text and in each table and figure.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Materials and Methods

Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference. This includes a description of the design, measurement and collection of data, type and source of subjects, inclusion and exclusion criteria and measures of outcome, number of subjects studied and why this number was chosen. Any deviation from the study protocol should be stated. Randomized controlled trials should adhere to the CONSORT guidelines that can be found at: http://www.consort-statement.org. Observational studies should also adhere to Strobe statement: http://www.strobe-statement.org/. Diagnostic accuracy studies should follow the Stard statement: http://www.stard-statement.org/. Systematic Reviews and Meta-Analyses should adhere to the PRISMA statement: http://www.prisma-statement.org/.

Statistical analysis

The statistical methods used should be relevant and clearly stated. Special or complex statistical methods should be explained and referenced. Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as P values, which fail to convey important information about effect size. Define statistical terms, abbreviations, and symbols. Specify the software used.

Units

Follow internationally accepted rules and conventions: use the internal system of units (SI).

Results

Results should be clear and concise. Results should be explained and illustrated by using Tables and Figures. Do not duplicate information contained in tables and figures.

Discussion

Discussion should directly relate to the results of the study and should explore their significance. Do not provide a general review of the topic.

Conclusions

The conclusions should provide a summary of the key results and discuss the appropriateness and impact of this original work.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references. Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

References

Ensure that every reference cited in the text is also present in the reference list (and vice versa). References should be numbered in the order they appear in the text. Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/. In the text, references should be cited using Arabic numerals enclosed in square brackets [1]. The last names and initials of all authors should be referred to if they are up to six, otherwise only the first six are referred, with et al following. References should also include full title and source information. Journal names should be abbreviated according to the standard in the Index Medicus. No periods should be placed at the end of abbreviations of the journal.

Journal article, up to 6 personal author(s):

Example: Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

Journal article, more than 6 personal author(s):

Example: Liaw S, Hasan I, Wade, V, Canalese R, Kelaher M, Lau P, et al. Improving cultural respect to improve Aboriginal health in general practice: a multi-perspective pragmatic study. Aust Fam Physician. 2015;44(6):387-92.

Journal article/ Issue with a supplement

Example: Bonda C, Sharma P, LaFaver K. Clinical reasoning: a 28 year-old woman with lower extremity spasticity and microcytic anemia. Neurology. 2015;85(2) Suppl:e11-4.

Electronic journal article:

Example: Poling J, Kelly L, Chan C, Fisman D, Ulanova M. Hospital admission for community-acquired pneumonia in a First Nations population. Can J Rural Med [Internet]. 2014 Fall [cited 2015 Apr 27];19(4):135-41. Available from: http://www.srpc.ca/14fal.html by selecting PDF link in table of contents.

Book, personal author(s):

Example: Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; c2012.

Book or pamphlet, organization as both author and publisher:

Example: College of Medical Radiation Technologists of Ontario. Standards of practice. Toronto: The College; 2011.

Book, editor(s):

Example: Kumar V, Abbas AK, Aster JC, editors. Robbins basic pathology. 16th ed. Philadelphia: Elsevier Saunders; c2013.

Poster presentation/session presented at a meeting or conference:

Example: Chasman J, Kaplan RF. The effects of occupation on preserved cognitive functioning in dementia. Poster session presented at: Excellence in clinical practice. 4th Annual Conference of the American Academy of Clinical Neuropsychology; 2006 Jun 15-17; Philadelphia, PA.

Tables

Tables should be typewritten, double-spaced, each one on a separate page and numbered consecutively with Arabic numerals in the order of their appearance in the text. Do not duplicate material presented in a figure. Tables should include a short but concise title. Tables should read vertically when possible. Place explanatory matter in footnotes, including any non-standard abbreviation. If data from another published or unpublished source are used, obtain permission and acknowledge fully.

Figure legends

Figure legends should be listed one after the other, as part of the main text, separate from the figure files. Each figure legend should have a brief title (in bold with figure number) followed by a description of each panel, and the symbols used. The statistical test used as well as the values of statistical significance (whether significant or not) should always be included in the figure legends. If a figure has been published previously, acknowledge the original source and submit written permission from the copyright holder to reproduce it. Authors will be required to pay for the extra cost of printing illustrations in color. However, there is an option to have their images in color in the electronic version of their manuscript and in grey scale in the printed version.

Figures

All figures for review should be submitted as a separate file in JPEG or TIFF format in grayscales or in RGB color mode with a resolution of at least 300 dpi. Number figures consecutively using Arabic numerals.

Photographs should be submitted as TIFF with a resolution of at least 300 pixels per inch; or Illustrator compatible EPS files with RGB color management or Photoshop or editable PDF files (grayscales or RGB).

Photographs of identifiable patients should be accompanied by written permission to publish from patient(s).

RGB figures will be presented in color in the electronic version and in grey scale in the printed version.

Ethical Considerations

An author should not publish manuscripts describing essentially the same research in more than one journal or primary publication. It must not be under consideration for publication elsewhere, and, if accepted, must not be published elsewhere in similar form, in any language. The International Committee of Medical Journal Editors has a full description about duplicate or redundant publication (http://www.icmje.org).

Authorship should be limited to those who have made a significant contribution to the conception, design, execution, or interpretation of the reported study.

The 'Achaiki Iatriki' editors endorse the principles of the Declaration of Helsinki and expect that all investigations involving humans will have been performed in accordance with these principles.

Authors should carefully protect patients' anonymity. Manuscripts reporting data from research conducted on humans must include a statement of assurance in the materials and methods section describing that: written informed consent was obtained from each patient included in the study and that the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

Do not use patients' names, initials, or hospital numbers, especially in illustrative material.

For animal experimentation reported in the journal, it is expected that investigators will have observed the Interdisciplinary Principles and Guidelines for the Use of Animals in Research, Testing, and Education issued by the New York Academy of Sciences' Adhoc Committee on Animal Research.

Disclosures: Conflict of interest

All authors are required to provide a Declaration of Interest Statement recognizing and disclosing financial and other conflicts of interest that might bias their work. Particularly, they disclose any actual or potential conflict of interest including any financial, activities, additional affiliations, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, their work. Further information at International Committee of Medical Journal Editors ("Uniform Requirements for Manuscripts Submitted to Biomedical Journals") -- February 2006

Disclosures: Financial disclosure

Authors are requested to identify who provided financial support for the conduct of the research and/or preparation of the article and to briefly describe the role of the sponsor(s), if any, in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the article for publication. If the funding source(s) had no such involvement then this should be stated.

Inform Consent

Patients have a right to privacy that should not be infringed without informed consent. Information such as patients' names, initials, or hospital numbers, should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent.

Identifying details should be omitted if they are not essential. Complete anonymity is difficult to achieve, however, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of patients is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic pedigrees, authors should provide assurance that alterations do not distort scientific meaning.

Further information at International Committee of Medical

Journal Editors ("Uniform Requirements for Manuscripts Submitted to Biomedical Journals") -- February 2006

Human and Animal Rights

Manuscripts reporting experiments using humans or animals must include a statement giving assurance that all humans or animals received human care and that study protocols comply with the institution's guidelines. When reporting experiments on human subjects, authors should indicate whether the procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. When reporting experiments on animals, authors should be asked to indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Further information at International Committee of Medical Journal Editors ("Uniform Requirements for Manuscripts Submitted to Biomedical Journals") -- February 2006

Copyright assignment

Upon acceptance of an article, authors will be asked to complete a copyright assignment indicating that exclusive copyright in the paper is assigned to the Publisher.

MANUSCRIPT PROCESSING AND REVIEW

Submission

Submission to ACHAIKI IATRIKI proceeds via email to achaiki.iatriki@gmail.com

Review process

Each manuscript submitted to ACHAIKI IATRIKI is assigned to a Section Editor who has expertise on the subject of the manuscript. The Section Editor initially evaluates the manuscript if it is appropriate and competitive for publication and sends the manuscript to 2-4 reviewers who are experts in the field.

PUBLICATION

Proofs

Proofs will be made available to the author(s) to be checked. It is the responsibility of the author(s) to make sure that the quality and accuracy of the manuscript, figures, and tables in the proofs is correct. At this stage, authors may make only minor corrections. Authors should return their proofs within 48 hours, by e-mail. At this point the author may order reprints, which are charged according to the number of reprints and the number of pages of the article.

Achaiki Iatriki