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# KUWAIT MEDICAL JOURNAL

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# Kuwait Medical Journal

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***Book***: Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical microbiology. 4th ed. St. Louis: Mosby; 2002.

***Book chapter***: Meltzer PS, Kallioniemi A, Trent JM. Chromosome alterations in human solid tumors. In: Vogelstein B, Kinzler KW, editors. The genetic basis of human cancer. New York: McGraw-Hill; 2002. p. 93-113.

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Review Article

# Thematic trends in Intensive Care Medicine publications from South Asia: A comparative bibliometric review from 2014 to 2024

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Kuwait Medical Journal 2026; 58 (1): 1 - 12

## ABSTRACT

Journals of South-Asian origin under the patronage of their respective academic societies in the specialty of Intensive Care have been publishing varied scientific content for almost three decades now. We aimed to evaluate the publication trends in a candidate representative journal of Intensive care published from South-Asia, using bibliometric analysis of author keywords. The Scopus database was searched for and the Indian Journal of Critical Care Medicine (IJCCM) dataset (eISSN number: 1998-359x) from 01 January 2014 to 15 September 2024 was downloaded. Similarly, we retrieved the corresponding data files of high impact journals - Critical Care Medicine and Critical Care. Visualization patterns, clustering and author keyword co-occurrence in these journals were obtained using VOSviewer®. A total of 2691 publications from South-Asian journals featured during the timeline were obtained comprising 1738 articles, 477 letters, 336 editorials, 100 reviews, 27 notes, 12 erratums and 1 conference paper, with Critical Care Medicine and Critical Care contributing 6370 and 4889 publications respectively. A limit in VOSviewer® of minimum author keyword occurrence of 10 yielded 113 keywords for IJCCM from total 4899 keywords. The thematic clustering of top 100 keywords across these journals outlined an adequate representation of contemporary topics at the South-Asian journal, at the same time providing useful insights into global-regional publication interests. The representative South-Asian journal of intensive care medicine in our bibliometric review exhibited considerable growth in diversity of ICU topics catered, albeit the need for focusing on unique opportunities for charting future path through improving altmetric indices for wider dissemination of knowledge.

**KEY WORDS:** bibliometrics, critical care medicine, intensive care unit, keyword, publication trends

## INTRODUCTION

The specialty of Intensive care medicine has undergone considerable evolution and improvement over the last two decades in South-Asia, particularly in the realms of clinical practice, research and publications. Professional societies, like the Indian Society of Critical Care Medicine (ISCCM); Pakistan Society of Critical Care Medicine and the Nepalese Critical Care Development Foundation pioneered by multi-disciplinary physicians played a pivotal role in fostering education and collaboration amongst the sprawling number of team members in this vast arena of knowledge[1,2].

Apart from providing the ICU physicians with a platform for communicating their research findings to the global community, official journals by these societies have also been at the forefront in publishing narrative reviews on specialised topical topics like the intensive care management of acute stroke[3], registry data on ICU admissions[4], clinical practice guidelines on antibiotic prescription policies in the ICU[5], nutrition in the ICU[6], organ donation management[7], renal replacement therapy[8], and prevention of hospital acquired infections[9]. Even in the challenging phase for the scientific community during an unprecedented global pandemic situation[10,11], the intensive care

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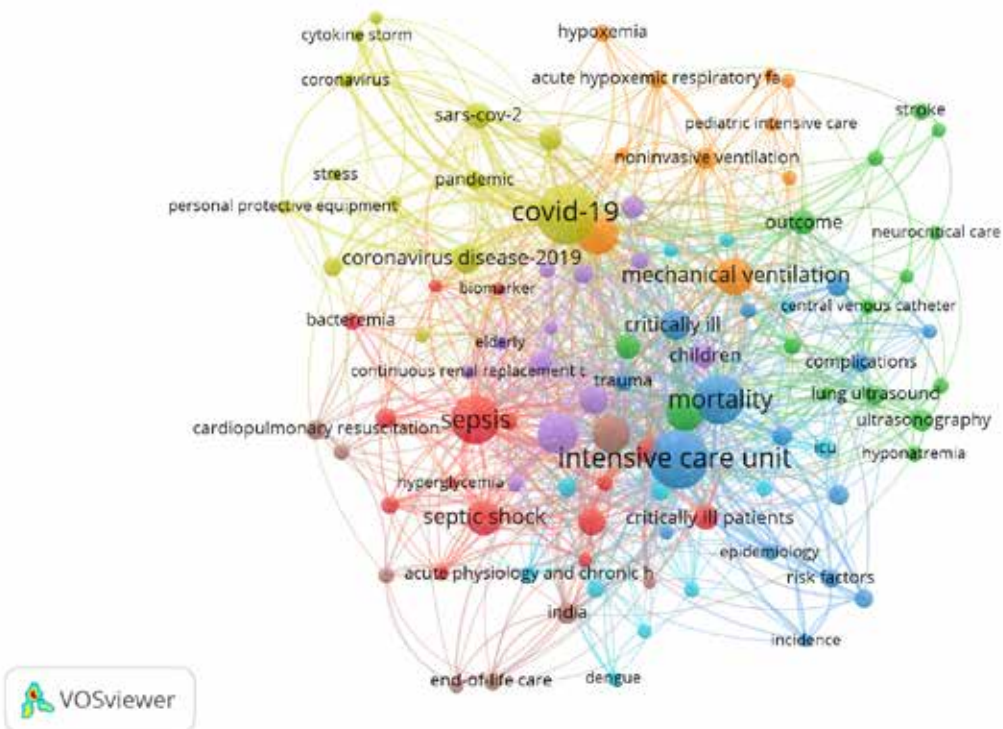
specialty journals from South Asia ensured a timely provision of the guidelines on ICU management of COVID-19 patients[12]. Having said that, given the sheer extent of the assorted articles published in those journals over the last decade, it is through a bibliometric analytic review that we can learn in detail of the broad themes dealt by them so far. The bibliometric tools can evaluate and enumerate large volumes of data, contributing worthwhile information beyond what a meta-analysis or systematic review has to offer on a prespecified topic[13]. Hence, we conducted a bibliometric study, with the primary objective to evaluate the publication trends in a representative candidate journal of intensive care published from South Asia, over the last decade using keyword co-occurrence analysis. We further resorted to a similar analysis for couple of other leading journals with high impact factors in the specialty of Intensive Care, aiming to compare the related keyword trends thereof.

## METHODS

The Scopus database was searched for the intensive care literature collection. Among the intensive care medicine journals published from South Asia, we chose the Indian Journal of Critical Care Medicine (IJCCM) as the representative candidate journal for our study, to ensure availability of a validated impact factor and PubMed indexing among the peers published in the region[2]. Using the advanced search option on Scopus, a dataset of all types of articles published in IJCCM (corresponding to eISSN number: 1998-359x) from the date range 01 January 2014 to 15 September 2024 was downloaded as a (.csv) file in Microsoft excel format. We additionally retrieved the dataset of two other high impact journals in the branch (Critical Care Medicine and Critical Care), for the same timeline using a search strategy with the corresponding ISSN numbers 1530-0293 and 1364-8535 respectively, in the Scopus database.

We employed the VOSviewer® version 1.6.20 (Leiden University Centre for Science and Technology, The Netherlands) to analyze the visualization patterns (network, overlay and density); clustering and the co-occurrence of keywords in each of the selected journals. A set limit in VOSviewer® of minimum author keyword occurrence of 10, yielded 113 keywords for IJCCM from an overall total of 4,899 keywords. With the same keyword occurrence limit, there were 326 keywords from a total of 7084 for Critical Care Medicine; and 164 from a total of 5288 author keywords for Critical Care during the study timeline. In order to ensure clarity of depiction within the sprawling number of keywords identified, we chose the 100 most frequently occurring author keywords with the highest co-occurrence linkage strength in VOSviewer®. Clustering of these 100 keywords based on the broad themes they deal with were identified for each of the studied journals.

Citation count of the articles published in the representative South Asian journal (IJCCM) was done from Scopus to identify the top 10 most cited articles during the study timeline. Altmetric indices of mentions in social media platforms, for the top



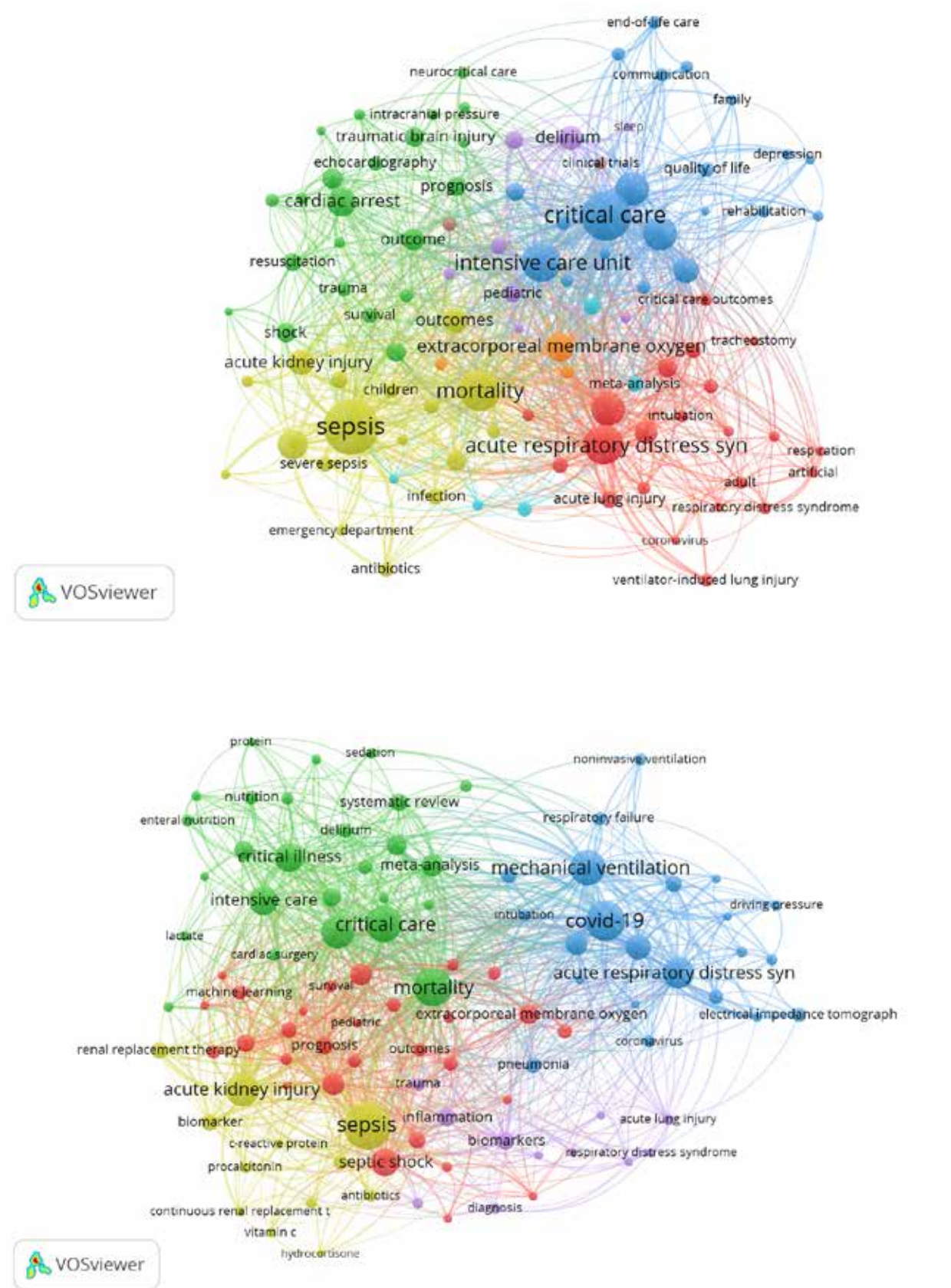



**Figure 1:** Network visualization analysis of most co-occurred 100 keywords with highest linkage strength in (A) Indian Journal of Critical Care Medicine (B) Critical Care Medicine (C) Critical Care.

10 cited articles we found, were elucidated from the Dimensions AI® database (Digital Science and Research Solutions Inc, 6 Briset St, London, UK). Google My Maps® (Amphitheatre Parkway, Mountain view, California 94043, USA) application was used to identify the global geographical distribution of origin of articles in the IJCCM.

Further, hotspot mapping of most frequently featured authors in this journal was found with the VOSviewer®. The number of articles per each category (editorial, original article, review, systematic review and letters) during the study timeline were also evaluated.

## RESULTS

We found a total of 2691 articles published in IJCCM during the study timeline, which included 1738 articles, 477 letters to the editor, 336 editorials, 100 review articles, 27 notes, 12 erratums and 1 conference paper. On the other end, Critical Care Medicine and Critical Care contributed 6370 and 4889 total articles, respectively.

Network visualization of the top 100 keywords based on their occurrence and linkage strength in VOSviewer®, for the last decade in each of the candidate journals we studied are as in Figure 1 (A, B and C). Size of each node in the network visualization indicates the number of times the corresponding keyword occurs and the thickness of link between the nodes signals the events of co-occurrences between these keywords. Keywords based on a common theme are color coded to a cluster as in Figure 1. Such thematic clustering of keywords and individual keywords in each of these clusters in the selected journals for this study are as in Table 1. There were 8 clusters each identified for IJCCM and Critical care medicine, whereas 5 clusters were found for Critical care.

The top 10 most cited papers in the representative South Asian journal for the last decade and their corresponding altmetric performance are as in Table 2. Geographic mapping of origin among articles published in IJCCM during this study timeline is illustrated in Figure 2. As per VOSviewer®, a total of 6914 authors were featured in IJCCM during the last decade. Hotspots mapping of authors with at least 10 publications in IJCCM during the study timeline outlined 82 authors (Figure [3A]); with a network linkage amongst 64 of them (Figure [3B]).

## LITERATURE REVIEW

In this study, we evaluated for the first time the publication trends within a South Asian journal of


**Figure 2:** Mapping of global author origin in the Indian Journal of Critical Care Medicine.

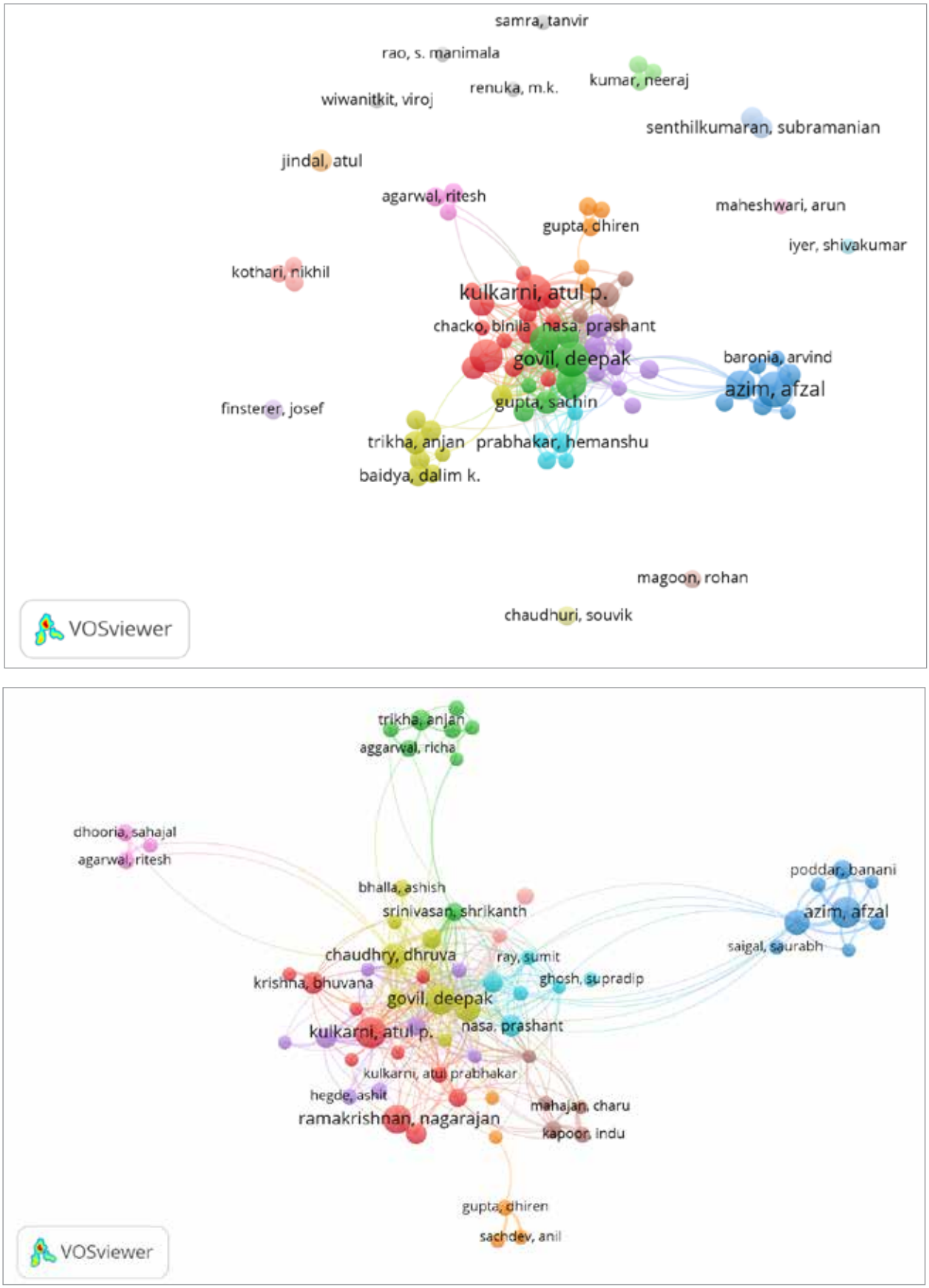



**Figure 3:** Author (with at least 10 in-house publications) network analysis of Indian Journal of Critical Care Medicine (A) without and (B) with network linkages.

specialty Intensive Care for the last ten years and compared it with two other high impact journals in intensive care, focusing descriptive analysis of co-occurrence of the author keywords, having utilized a bibliometric software. We chose the author keywords with the highest number of occurrences along with the highest linkage in the selected intensive care journals, as to be able to churn out the most debated-discussed themes in this arena. The merit of this concept is nested in the very fact that the keywords are useful snippets of information on the content-theme of any article wherein authors execute utmost caution in the selection of keywords for them to be truly reflective of the core subject matter of their manuscript.

As for our choice of the Scopus database for search and preparation of our metadata, IJCCM has been indexed with Scopus since 2004[2] and all the IJCCM publications during our study timeline stand inclusive to the database. We do acknowledge that the IJCCM home webpage offers a glimpse of its' article and author metrics[2]. A web link here leads to a domain named as *"lens.org"* wherein one can find certain amount of bibliometric data. Owing to the need for uniformity of comparison across the journals, a common Scopus database was chosen, alongside the fact that a comprehensive keyword-based study could also not have been contemplated using the metadata related to the *"lens.org"* web domain.

We chose two among the critical care specialty journals with the highest impact factor[14] for keyword co-occurrence comparison. Certain journals like the American Journal of Respiratory and Critical Care Medicine, Chest and the Lancet Respiratory Medicine, though among the highest impact factor category herein, were not opted for, as they propose pulmonology topics more often than critical care. As the specialty of critical care continues to evolve at a rapid and dynamic pace, a database analysis of this specialty journals helps intensivists understand the evolving trends of interdisciplinary research herein[15-17].

We identified that a vast majority amongst the top 100 keywords in the candidate South Asian journal during the last decade, frequently occurred also in the other two high-impact international critical care journals (Figure 1 [A, B and C]). This bespeaks that IJCCM has handled in detail the widely acclaimed and sought after topics in the branch (Table 1). Notably, a few keywords like scrub typhus, dengue, thrombocytopenia and poisoning uniquely occurred frequently in the IJCCM. Griffith *et al*[18] attempted to elucidate the stretch of organ dysfunction (based on Sequential Organ Failure Assessment score at ICU admission) and its relation to mortality in a cohort of 116 adult scrub typhus patients in an ICU, retrospectively. Majority of patients (88%) in this cohort were on mechanical ventilation with 24% mortality outcome. A multivariate analysis of factors affecting survival found Acute Physiology and Chronic Health Evaluation II score (Odds Ratio [OR]=1.2, P<0.01) and duration of pyrexia (OR=0.75, P=0.01) to be significantly related. However, despite frequent multi-organ dysfunction and higher illness severity, scrub typhus patients had lesser than predicted mortality. Kar *et al*[19] prospectively studied a pertinent neurologic manifestation of Scrub Typhus - Acute encephalitic syndrome, among 20 patients over a 9 months' timeline and infer the importance of early involvement of multi-disciplinary teams and early initiation of Doxycycline therapy.

Dengue fever, much in congruence, is a vector-borne viral illness spread by *Aedes albopticus* and *Aedes egypti* mosquitoes, with a high prevalence in tropical areas[20,21]. Padyana *et al* [22] in their retrospective study on the clinical variables and outcomes in patients with dengue fever requiring ICU admission depicted hyperlactatemia, raised liver transaminase, low blood pressure and low P/F ratios as factors predictive of mortality. In addition, Pothapregada *et al* in their research cohort of children admitted to ICU with dengue fever brought to light hepatomegaly (OR 4.3 [2.37-7.89]); ascites (OR 6.6 [2.13-21.01]; hyponatremia (OR 25 [3.22-194.8]; oliguria (OR 4.3 [9.57-67.72] and thrombocytopenia (OR 11.3 [2.48-51.91] as significant risk factors (P<0.001) for shock[23]. We acclaim the continued impetus over the last decade, given by IJCCM to tropical fevers[24] and the management of the associated peculiar complications in the ICU[25,26]; and attribute this to the frequent presence of such unique keywords in corresponding clusters (Table 1). Herein, we assume that regional distribution and prevalence of disease related to intensive care was well catered to and described by IJCCM for the benefit of the practicing critical care physicians in South Asia.

Poisoning due to organophosphate (OP) compounds, a common ingredient in pesticides, is also more prevalent in the developing nations[27]. Peter *et al* attempted a time-based classification of symptoms after OP poisoning in their study of 452 articles on this subject[28]. The authors found differential early (up to 24 hours); intermediate (24 hours to 2 weeks); and late (>2 weeks) sequelae with di-methyl and di-ethyl group of OP compounds. Subsequent research work by Jha *et al* marked a significant correlation between low RBC-cholinesterase levels and requirement of atropine doses among a prospective group of 60 adult patients presenting to the ER with OP poisoning[29]. Poisoning due to phosphine gas from aluminium phosphide, which is a common constituent in fumigant pesticides, is also a common toxidrome seen widely in agricultural predominant localities in India. The research work by Agarwal *et al* provides scientific insights about

**Table 1:** Illustration of independent clusters, and keywords identified within each cluster in the selected journals, as per VOSviewer®

| IJCCM | Critical Care Medicine | Critical Care |
|---|---|---|
| **Cluster 1 (n=15)** | **Cluster 1 (n=22)** | **Cluster 1 (n=29)** |
| Antibiotics, Bacteremia, Biomarker, Biomarkers, Critical illness, Critically ill patients, Enteral nutrition, Guidelines, Hyperglycemia, Intensive care units, Procalcitonin, Prognosis, Randomised control trial, Sepsis, Septic shock | Acute lung injury, Acute respiratory distress syndrome, Acute respiratory failure, Adult, Artificial, Corona virus, Corona virus disease 2019, Covid-19, Critical care outcomes, Intubation, Mechanical ventilation, Noninvasive ventilation, Obesity, Pneumonia, Prone position, Respiration, Respiratory distress syndrome, Respiratory failure, Respiratory insufficiency, SARS-CoV2, Tracheostomy, Ventilator-induced lung injury | Angiotensin 2, Artificial intelligence, Blood pressure, Cardiac arrest, Cardiogenic shock, Cardiopulmonary resuscitation, Echocardiography, ECMO, Epidemiology, Extracorporeal membrane oxygenation, Fluid responsiveness, Fluid therapy, Hypotension, Machine learning, Microcirculation, Norepinephrine, Out-of-hospital cardiac arrest, Outcome, Outcomes, Pediatric, Pediatrics, Prediction, Prognosis, Prognostication, Resuscitation, Septic shock, Shock, Survival, Vasopressor |
| **Cluster 2 (n=15)** | **Cluster 2 (n=22)** | **Cluster 2 (n=26)** |
| Acute respiratory failure, Central venous catheter, Critical care, Deep vein thrombosis, Echocardiography, Emergency department, Hyponatremia, Lung ultrasound, Neurocritical care, Optic nerve sheath diameter, Outcome, Pulmonary embolism, Stroke, Thrombolysis, Ultrasonography | Biomarker, Cardiac arrest, Cardiopulmonary resuscitation, Coma, Echocardiography, Guidelines, Heart arrest, Hypothermia, Inflammation, Intracranial pressure, Neurocritical care, Outcome, Prognosis, Resuscitation, Shock, Stroke, Subarachnoid hemorrhage, Survival, Therapeutic hypothermia, Trauma, Traumatic brain injury, Vasopressor | Cardiac surgery, Critical care, Critical illness, Critically ill, Delirium, Emergency department, Enteral nutrition, Hyperoxia, ICU, Intensive care, Intensive care unit, Intensive care units, Lactate, Meta-analysis, Metabolism, Mortality, Nutrition, Parenteral nutrition, Protein, Quality of life, Randomized control trial, Rehabilitation, Sedation, Systematic review, Traumatic brain injury, Weaning |
| **Cluster 3 (n=15)** | **Cluster 3 (n=21)** | **Cluster 3 (n=22)** |
| Complications, Critically ill, Delirium, Epidemiology, Hypoxia, Incidence, Intensive care unit, Intubation, Mortality, Pediatric, Risk factors, Tracheostomy, Trauma, Ultrasound, Ventilator- associated pneumonia | Child, Communication, Critical care, Critical illness, Depression, End-of-life care, Ethics, Family, Hospital mortality, Intensive care, Intensive care unit, Intensive care units, Outcome assessment, Palliative care, Patient safety, Post-intensive care syndrome, Post-traumatic stress disorder, Quality improvement, Quality of life, Rehabilitation, Risk factors | Acute respiratory distress syndrome, Acute respiratory failure, ARDS, Corona virus, Coronavirus disease 2019, Corticosteroids, Covid-19, Driving pressure, Electrical impedance tomography, Intubation, Mechanical power, Mechanical ventilation, Noninvasive ventilation, Pneumonia, PEEP, Prone position, Prone positioning, Respiratory failure, Respiratory mechanics, SARS-COV-2, Ventilator-associated pneumonia, Ventilator-induced lung injury |
| **Cluster 4 (n=13)** | **Cluster 4 (n=17)** | **Cluster 4 (n=12)** |
| Communication, Corona virus, Corona virus disease 2019, Corona virus disease-2019, Covid-19, Cytokine storm, Healthcare workers, Interleukin-6, Pandemic, Personal protective equipment, SARS-CoV2, Stress, Tocilizumab | Acute kidney injury, Antibiotics, Biomarkers, Children, Critically ill, Diagnosis, Emergency department, Epidemiology, Infection, Length of stay, Mortality, Organ dysfunction, Outcomes, Renal replacement therapy, Sepsis, Septic shock, Severe sepsis | Acute kidney injury, Antibiotics, Biomarker, C-reactive protein, Continuous renal replacement therapy, Hydrocortisone, Infection, Pharmacokinetics, Procalcitonin, Renal replacement therapy, Sepsis, Vitamic C |
| **Cluster 5 (n=12)** | **Cluster 5 (n=8)** | **Cluster 5 (n=11)** |
| Acute kidney injury, Children, Continuous renal replacement therapy, Elderly, Extracorporeal membrane oxygenation, Hemodialysis, Pediatric intensive care unit, Pneumonia, Poisoning, Renal replacement therapy, Sequential organ failure assessment score, Shock | Cardiac surgery, Delirium, Morbidity, Pediatric, Pediatric critical care, Pediatrics, Sedation, Sleep | Acute lung injury, Adult, Biomarkers, Cytokines, Diagnosis, Immunosuppression, Inflammation, Personalised medicine, Precision medicine, Respiratory distress syndrome, Trauma |
| **Cluster 6 (n=12)** | **Cluster 6 (n=5)** | |
| Acute physiology and chronic health evaluation 2, Covid- 19 infection, Covid-19 mortality, Critically ill adults, Dengue, ICU, ICU mortality, Mortality prediction, Outcomes, Pregnancy, Scrub typhus, Thrombocytopenia | Corticosteroids, Incidence, Meta-analysis, Systematic review, Ventilator-associated pneumonia | |

| Cluster 7 (n=10) | Cluster 7 (n=3) |
|---|---|
| Acute hypoxemic respiratory failure, Acute respiratory distress syndrome, Analgesia, Hypoxemia, Mechanical ventilation, Noninvasive ventilation, Pediatric intensive care, Respiratory failure, Sedation, Ventilation | Cardiogenic shock, Extracorporeal life support, Extracorporeal membrane oxygenation |
| **Cluster 8 (n=8)** | **Cluster 8 (n=2)** |
| Cardiac arrest, Cardiopulmonary resuscitation, End-of-life care, India, Intensive care, Knowledge, Palliative care, Predictors | Clinical trials, Machine learning |

oxidative stress, etio-pathogenic mechanisms to the peculiar symptoms, through the serial analysis of serum levels of catalase, lactate, super-oxide dismutase and glutathione peroxidase[30].

To that end, we found some keywords to be quite distinctive to the high impact journals we studied. Topics on "quality improvement", "quality of life", "hypothermia/therapeutic hypothermia", "patient safety", "machine learning", "post-intensive care syndrome", "subarachnoid hemorrhage" and "ethics" were the notable ones in the Critical Care Medicine. Whereas, topics like "nutrition", "driving pressure", "electrical impedance tomography", "mechanical power", "artificial intelligence", "vitamin C", "precision medicine", "personalized medicine" and "hyperoxia" were peculiar to the top 100 author keywords in the Critical Care.

Personalized medicine or precision medicine is a medical model to tailor stratified medical decisions, interventions and practices to an individual patient based on their characteristic risk of disease or response to treatment[31,32]. This novel concept, which was initially unrolled for genome targeted therapies in oncology and rheumatology, has gained considerable attention in critical care. A targeted pre-clinical workup can identify patients with inflammatory phenotypes predisposing to thrombocytopenia-associated multiorgan failure; macrophage activation syndrome; and sequential multi-organ failure. Seymour *et al* elucidated that identifying such putative endotype based targets in sepsis therapy, though challenging, can pave the way towards disease modifying drug therapies[33]. Though precision medicine enacts a 'personalized' approach to each patient based on sub-phenotype, intensivists need to work out ways on how such prescriptions can expand from the research setting to daily clinical care[34].

Journal-centric importance to the physiology of breathing and mechanical ventilation through research over this timeline on topics like driving pressure[35]; respiratory mechanics[36]; and mechanical power[37] is well-evidenced by the presence of these keywords in cluster 3 of Critical Care (Table 1). Among patients on mechanical ventilation, dynamic evaluation of ventilation distribution; lung pulsatility and perfusion, non-invasively without radiation exposure has been attempted with the advent of electrical impedance tomography (EIT)[38]. Assessment of EIT plethysmogram and ventilation map can aid evaluation of ventilation-induced lung injury (collapse or over distension); pneumothorax and ventilation dys-synchrony. The popularity of this tool in surveillance of acute respiratory distress syndrome patients; and the evolving research emphasis is also evident from cluster 3 of Critical care. Similarly, integration of artificial intelligence (AI) into patient care in the ICU is also gaining momentum (Cluster 1 of Critical Care). AI can precisely diagnose congestive heart failure from other causes of lung infiltrates and lesions in a computed tomography scan in traumatic brain injury (TBI) without manual reading[39,40]. The research work by Nemati *et al* concluded that onset of sepsis can be predicted up to 12 hours (Area under receiver operating characteristic curve=0.83) prior to clinical recognition with the use of an AI sepsis expert algorithm[41]. Machine learning models analyzing patient demographics and serial vital signs far outperformed logistic regression analysis, in the prediction of clinical deterioration; requirement of ICU admission and mortality in a large cohort of patients involved in the study by Churpek *et al*[42]. It is worthwhile to note that personalized medicine and machine learning integration to clinical care are emerging frontiers in the ICU, and has largely been dealt with by Critical care medicine and Critical care.

At the same time, the emphasis on neuro-critical care research related to subarachnoid haemorrhage (SAH), TBI and targeted temperature management given by Critical care medicine is evident from the corresponding keyword cluster 2. Rass *et al* validated age, admission acute physiology score, Hess and Hunt grade, SAH early brain edema score and presence of

**Table 2:** Top 10 cited* articles in the representative candidate journal of Intensive Care from South-Asia (Indian Journal of Critical Care Medicine) during 2014 to 2024, and their corresponding altmetric# performance/mentions data

| Rank | Article | Article category$ | Citations as per Scopus | Citations as per Google Scholar | Altmetric performance and mentions |
|---|---|---|---|---|---|
| 01 | Khasne RW, Dhakulkar BS, Mahajan HC, Kulkarni AP. Burnout among Healthcare Workers during COVID-19 Pandemic in India: Results of a Questionnaire-based Survey. Indian J Crit Care Med. 2020;24(8):664-671. doi:10.5005/jp-journals-10071-23518. | Original research | 185 | 393 | 466 Mendeley<br>5 News<br>1 Blog |
| 02 | Peter JV, Sudarsan TI, Moran JL. Clinical features of organophosphate poisoning: A review of different classification systems and approaches. Indian J Crit Care Med. 2014;18(11):735-745. doi:10.4103/0972-5229.144017. | Review | 178 | 347 | 465 Mendeley<br>21 Twitter<br>12 Wikipedia<br>2 News<br>1 Facebook<br>1 Blog |
| 03 | Mehta Y, Gupta A, Todi S, Myatra S, Samaddar DP, Patil V, et al. Guidelines for prevention of hospital acquired infections. Indian J Crit Care Med. 2014;18(3):149-163. doi:10.4103/0972-5229.128705. | Guidelines | 141 | 375 | 715 Mendeley<br>12 Twitter<br>7 Wikipedia<br>1 Facebook |
| 04 | Rapsang AG, Shyam DC. Scoring systems in the intensive care unit: A compendium. Indian J Crit Care Med. 2014;18(4):220-228. doi:10.4103/0972-5229.130573 | Review | 136 | 311 | 265 Mendeley<br>5 Twitter |
| 05 | Jose S, Dhandapani M, Cyriac MC. Burnout and Resilience among Frontline Nurses during COVID-19 Pandemic: A Cross-sectional Study in the Emergency Department of a Tertiary Care Center, North India. Indian J Crit Care Med. 2020;24(11):1081-1088. doi:10.5005/jp-journals-10071-23667 | Original research | 104 | 233 | 337 Mendely<br>1 Twitter<br>1 News |
| 06 | Dasgupta S, Das S, Chawan NS, Hazra A. Nosocomial infections in the intensive care unit: Incidence, risk factors, outcome and associated pathogens in a public tertiary teaching hospital of Eastern India. Indian J Crit Care Med. 2015;19(1):14-20. doi:10.4103/0972-5229.148633. | Research article | 101 | 305 | 328 Mendeley<br>1 Google+<br>1 Twitter |
| 07 | Chugh C. Acute Ischemic Stroke: Management Approach. Indian J Crit Care Med. 2019;23(Suppl 2):S140-S146. doi:10.5005/jp-journals-10071-23192. | Review | 96 | 213 | 989 Mendeley<br>2 News<br>1 Twitter |
| 08 | Saghaleini SH, Dehghan K, Shadvar K, Sanaie S, Mahmoodpoor A, Ostadi Z. Pressure Ulcer and Nutrition. Indian J Crit Care Med. 2018;22(4):283-289. doi:10.4103/ijccm.IJCCM_277_17 | Review | 95 | 245 | 590 Mendeley<br>5 News<br>3 Wikipedia<br>1 Twitter<br>1 Policy source |
| 09 | Divatia JV, Amin PR, Ramakrishnan N, Kapadia FN, Todi S, Sahu S, et al. Intensive Care in India: The Indian Intensive Care Case Mix and Practice Patterns Study. Indian J Crit Care Med. 2016;20(4):216-225. doi:10.4103/0972-5229.180042 | Research article | 94 | 146 | 144 Mendeley<br>3 Twitter<br>1 News |
| 10 | Javali RH, Krishnamoorthy, Patil A, Srinivasarangan M, Suraj, Sriharsha. Comparison of Injury Severity Score, New Injury Severity Score, Revised Trauma Score and Trauma and Injury Severity Score for Mortality Prediction in Elderly Trauma Patients. Indian J Crit Care Med. 2019;23(2):73-77. doi:10.5005/jp-journals-10071-23120 | Original article | 91 | 176 | Nil |

(*Data from Scopus was primarily used to rank the citations, Google scholar based citations rank may differ; #Altmetric data is obtained from Dimensions AI®; $Article category is as classified by the journal at the time of publication)

haemorrhage as risk factors associated with prolonged mechanical ventilation in a cohort of 297 aneurysmal SAH cases[43]. Cerebral autoregulation indices like a high pressure reactivity index with a low cerebral perfusion pressure were found to be associated with long term sequelae like delayed cerebral ischemia in the studies by Gaasch *et al* and Weiss *et al* respectively[44,45]. There has been commendable efforts to extend the beneficial cellular metabolism lowering effects of therapeutic hypothermia in scenarios like acute stroke;

SAH and TBI, though promising clinical effects have been found for mild hypothermia (33 to 37.5 °C) only among post-cardiac arrest survivors in coma[46-48].

In our study at a keyword co-occurrence preset limit of 10 in VOSviewer®, Critical care medicine had the highest number of 7084 keywords from 6370 publications. The high linkage strength we found to peculiar keywords like "ethics", "post-intensive care syndrome", "quality of life", "quality improvement" and "patient safety" nonetheless, reiterates the inherently dynamic nature of critical care literature[17].

In general, we discovered that the articles published at the IJCCM in the last decade, irrespective of the type of article, received a good number of citations (Table 2). The wide spread origin of authors from more than 70 countries for articles in the IJCCM, as evident from the geographic mapping (Figure 2), is a testimony to the growing popularity and readership of intensive care medicine journals from South Asia. The open access to full text policy of the journal[49,50] seems to have further aided the ready availability to intensivists worldwide and that could have contributed to the beneficence of citations and wider author origin. Beyond simple exchange of information, co-authorship network (Figure 3A and B) signify co-operative structure and collaborative behaviour among researchers and organisations featured in IJCCM over the last decade.

Apart from scholarly citations, altmetrics track the online attention received by an article in various media and social media platforms. In our study, we found that the top cited articles in IJCCM fared modest in such domains in comparison to articles elsewhere[51]. We suggest that authors and publishers at the South Asian intensive care medicine journals shall focus on improving the representation of future articles in social media platforms, in line with the novel age policies on dissemination of knowledge.

Our study had a few limitations to mention. Firstly, we chose all types of articles in the three candidate journals we evaluated. Frequently debated themes represent considerably as letters and correspondences in all academic journals and a repetition of keywords is a possibility here. Secondly, VOSviewer® evaluates each keyword independently premised on constituent 'characters', irrespective of the meaning conveyed. A manual exclusion of keywords with the same meaning is a resolving option here, however this is at the cost of identifying the top 100 keywords based on linkage strength. Bibliometric softwares like Gephi® and Leximancer® can address this limitation to a certain extent. Thirdly, total number of citations an article received vary when evaluated in different databases (Scopus versus Google scholar). Fourthly, it was not a part of our study to evaluate the funding agencies or patents received for any of the articles under analysis.

## CONCLUSION

In this index bibliometric study based on keyword co-occurrence in a representative candidate South Asian journal on specialty Intensive Care, we conclude that those fared well to publish contemporary issues in critical care, at the same time focused also on diseases and toxidromes prevalent in tropical regions. Precision medicine, quality improvement, patient safety, machine learning and neuro-intensive care happened to emerge as the certain niche areas that future publications from South Asia can refine its focus upon. Needless to say, improving the altmetric indices of such published articles can have far reaching benefits on the readership and dissemination of knowledge to the worldwide intensive care medicine fraternity.

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Original Article

# The expression pattern of 5-methylcytosine regulators and NSUN2 predicting oral squamous cell carcinoma prognosis by inhibiting immune reaction

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## ABSTRACT

**Objective:** RNA modification of 5-methylcytosine (m5C) has been discovered in diverse kinds of RNA and plays a vital role in gene regulation and cellular functions; however, how m5C regulates oral squamous cell carcinoma (OSCC) progression remains unclear.
**Design:** Bioinformatics analysis and validation
**Setting:** Stomatological Hospital
**Subjects:** Transcriptome and clinical data of OSCC samples (including 201 tumors and 18 normal samples) were downloaded from The Cancer Genome Atlas.
**Interventions:** Not applicable
**Main Outcome Measures:** Subjected to analyze m5C regulators expression and prognostic value. GSEA and immune infiltration analysis were conducted. Finally, NSUN2 expression in OSCC cell lines was validated by western blot.
**Results:** Of the 21 m5C regulators, 15 were significantly up-regulated (including NSUN2) in tumor tissue and only two were down-regulated, compared to normal tissue. Furthermore, among the 17 m5C regulators, univariate and multivariate cox regression analyses revealed that only NSUN2 was an independent prognostic risk factor for OSCC. GSEA revealed that pathways associated with immune response were enriched in the NSUN2-low expression group and pro-tumor pathways were enriched in the NSUN2-high expression group. Moreover, NSUN2 negatively correlated with active immune cell type, including $CD8^+$ T cells, B cells, NK cells, monocyte and gamma delta T cells, but positively correlated with myeloid-derived suppressor cells. NSUN2 expression was up-regulated in seven OSCC cell lines relative to normal cells.
**Conclusion:** In conclusion, m5C modification was active in OSCC tumor tissues and NSUN2 was a prognostic marker of OSCC, possibly by inhibiting immune reaction against tumor.

**KEY WORDS:** 5-methylcytosine, immune infiltration, NSUN2, oral squamous cell carcinoma

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) represents tumor originating from mucosa covering lip, tongue, bucca and gingiva, palate and floor of mouth. The incidence rate of OSCC is not low, with 350,000 new cases and 170,000 deaths in 2018[1]. Smoking, alcohol, betel quid chewing, HPV infection and other risk habits were common risk factors for OSCC tumorigenesis[2]. In addition, genetic factors (such as TP53, CDKN2A, PIK3CA, HRAS and NOTCH1 mutation) identified by next generation sequencing also proved to play an important role in OSCC occurrence[3,4]. Despite advances in surgery, chemotherapy and radiotherapy, especially targeted therapy, the prognosis of OSCC is not satisfying as the 5-year survival rate is still less than 50%[5]. Therefore, it is urgent to explore novel molecular mechanism underlying OSCC for new drug design.

RNA modifications played a vital role in gene regulation and cellular functions[6], and proved to be involved in many diseases, such as neurological disorder[7], immune dysregulation[8] and cancer[9].

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m6A was the most widely investigated type of RNA modification. Meanwhile, other types of RNA modification including N1-methyladenosine (m1A), 5-methylcytosine (m5C), 5-hydroxymethyl cytosine (hm5C), N7-methylguanosine (m7G), and pseudopurine (C) have also been identified[10,11]. Among them, m5C has attracted more attention.

The m5C has been discovered in diverse kinds of RNA (including ribosomal RNA, transfer RNA and messenger RNA) by transcriptome-wide mapping approaches[12]. Proteins involved in m5C consist of methyltransferases (writers), demethylases (erasers) and binding proteins (readers). The m5C writers include NSUN1-7, DNMT1, DNMT2, DNMT3A, and DNMT3B, m5C "erasers" consist of TET1-3 and ALKBH1, and m5C "readers" mainly consist of YTHDF2, ALYREF and YBX1[13,14]. The m5C participates in initiation, progression and prognosis of diverse cancers[15-17]. Recently, m5C has been revealed to affect head and neck squamous cell carcinoma (HNSCC) progression and predict patient's survival[18,19]. However, there are few research exclusively focused on m5C regulators role in OSCC, as HNSCC consists of not only OSCC, but also other cancers.

Therefore, this study was aimed to explore the expression pattern of m5C regulators and investigate the role of key gene of m5C regulator in OSCC through The Cancer Genome Atlas (TCGA) database, GSEA, immune infiltration pattern analysis, and western blot (WB) validation. This study will provide a basis for understanding the molecular mechanisms of OSCC from the perspective of m5C and provide new potential markers for the prognosis of OSCC patients.

## MATERIALS AND METHODS

### OSCC dataset acquisition and processing

Transcriptome and clinical data of OSCC samples (including 201 tumors and 18 normal samples) were downloaded from TCGA website (https://portal.gdc.cancer.gov/), anatomic sites of 201 tumors included tongue and floor of mouth, which belonged to TCGA-HNSC Program. Detailed clinical data were provided as Table S1. Gene expression data was downloaded as HTseq-FPKM form and then Ensemble ID of gene were transformed into gene name according to reference genome GRCh38.p12. The average of gene expression was calculated when there were more than 1 identical gene name. Single gene expression data was extracted and merged with clinical data to analyze its expression between tumor and normal tissues, then univariate and multivariate cox analysis were carried out. The above analysis was carried out by R 4.1.1 software utilizing limma package and survival package.

**Table 1:** Expression of M5C regulators between tumor and normal tissues

| Classification | Gene name | Tumor vs normal tissue (mRNA level) | Statistical significance |
|---|---|---|---|
| Writers | DNMT1 | Up-regulated | Yes |
| | DNMT2 | Up-regulated | Yes |
| | DNMT3A | Up-regulated | Yes |
| | DNMT3B | Up-regulated | Yes |
| | NSUN | Down-regulated | Yes |
| | NSUN2 | Up-regulated | Yes |
| | NSUN3 | Up-regulated | Yes |
| | NSUN4 | Up-regulated | Yes |
| | NSUN5 | Up-regulated | Yes |
| | NSUN6 | Up-regulated | No |
| | NSUN7 | Down-regulated | Yes |
| | TRDMT1 | Up-regulated | No |
| | TRM4A | Up-regulated | Yes |
| | TRM4B | Up-regulated | Yes |
| Erasers | TET1 | Up-regulated | No |
| | TET2 | Down-regulated | No |
| | TET3 | Up-regulated | Yes |
| | ALKBH1 | Up-regulated | Yes |
| Readers | ALYREF | Up-regulated | Yes |
| | YTHDF2 | Up-regulated | Yes |
| | YBX1 | Up-regulated | Yes |

### Gene Set Enrichment Analysis (GSEA)

GSEA 4.2.3 software was used to conduct this analysis. Two gene sets databases (h.all.v7.5.1.symbols.gmt and c2.cp.kegg.v7.5.1symbols.gmt) were chosen to conduct hallmark and KEGG analysis, number of permutations were 1000. Samples were divided into high group and low group according to the median value of NSUN2 expression, and low NSUN2 expression group was set as control. Other parameters were set as default. Gene set with $|NES| > 1$, NOM p-val <0.05 and FDR q-val <0.25 was rendered as statistically significant enrichment.

### Immune infiltration analysis

To analyze the relationship between NSUN2 gene expression and immune infiltration pattern, TIMER2.0 database (http://timer.cistrome.org/) was selected to conduct immune infiltration estimation. The partial Spearman's correlation was adopted to perform this association analysis. Other parameters were set as default.

### Cell culture

Two normal oral keratinocyte cell lines (human oral keratinocyte (HOK) and dysplasia oral keratinocyte (DOK)), and 7 OSCC cell lines (CAL27, CAL33, HSC3, HSC6, SCC9, SCC15, SCC25) and 1 cell line of pharyngeal squamous cell carcinoma (Fadu) were cultured. Normal oral keratinocyte (NOK), CAL27, CAL33, HSC3 and HSC6 were cultured in high glucose DMEM (Gibco, USA) supplemented

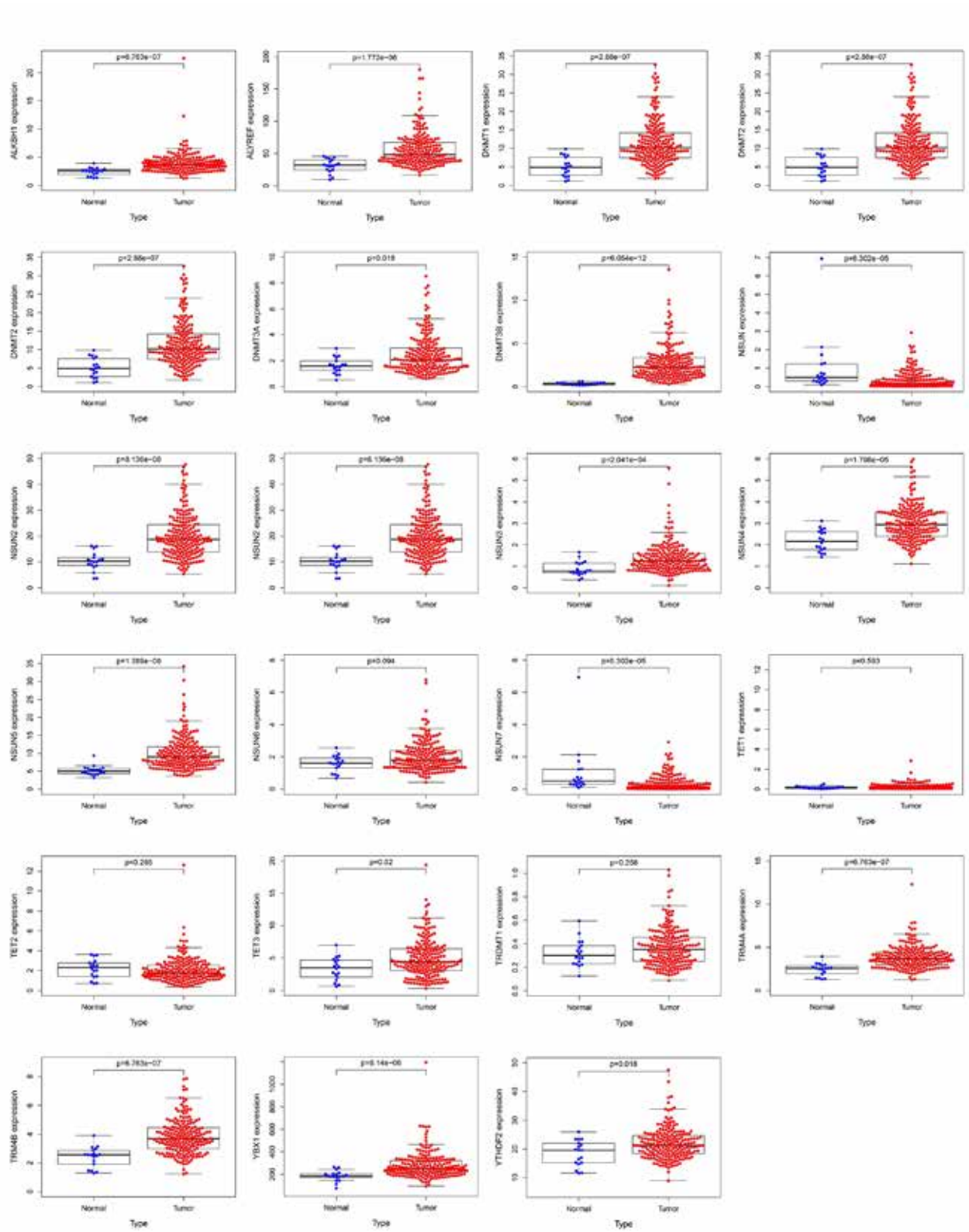


**Figure 1: m5C regulators differentially expressed between tumor and normal tissues in OSCC.** All m5C regulators mRNA expression between 201 tumor samples and 18 normal tissues of OSCC were analyzed. Among these regulators, 15 genes were significantly up-regulated in tumor samples, two genes (NSUN, NSUN7) were significantly up-regulated in tumor samples, and three genes (TET1, TET2, TRDMT1) expression were not significantly different.

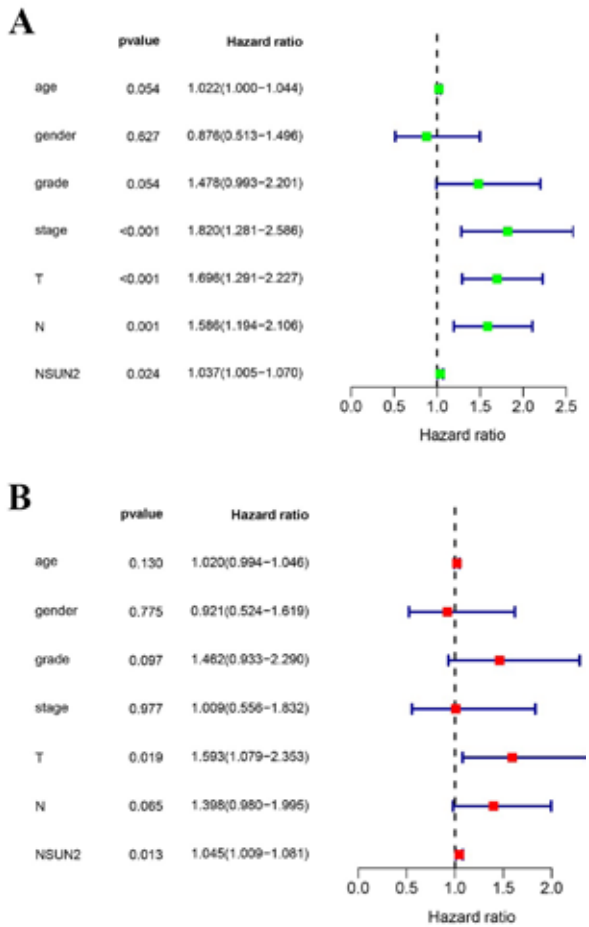


**Figure 2: NSUN2 was an independent risk factor for OSCC.** (A) Univariate cox analysis showed that NSUN2 was a risk factor for OSCC prognosis. In addition, clinical parameters (Stage, T and N status) were also risk factors. (B) Multivariate cox analysis showed that NSUN2 and T status were two independent risk factors for OSCC.

with 10% fetal bovine serum (FBS, Gibco, USA); SCC9, SCC15, SCC25 were cultured in DMEM/F12 (Gibco, USA) supplemented with 10% FBS and 4000 ng/mL hydrocortisone (Solarbio, China); HOK and Fadu were cultured in MEM (Gibco, USA) supplemented with 10% FBS; DOK were cultured in high glucose DMEM supplemented with 10% FBS and 5 μg/mL hydrocortisone. Cells were cultured in 37 °C and 5% $CO_2$ atmosphere.

### WB validation

Cells were lysed in RIPA (Beyotime, China) containing PMSF (1:100, Beyotime, China) for 30 min for total protein extraction after they were washed with precooled PBS 3 times. Protein quantification was carried out by BCA protein assay kit (Beyotime, China) and denaturation in SDS-PAGE Buffer (CWBIO, China) immediately. 10% SDS-PAGE gel was used to perform electrophoresis and then transferred to PVDF membrane (Millipore, USA), membrane were blocked with 5% skim milk (BD, USA) to reduce non-specific combination and then incubated with primary antibody NSUN2 (#DF12103, AffinityBiosciences, USA ) and GAPDH (#EM32010, EMRR, China) at 4 ℃ for 16 hours, then incubated with secondary antibody (#EM 35110, EMRR, China) in room temperature for 1 hour after washing with TBST (CWBIO, China) 3 times. Finally, membranes were exposed by using Ultra hypersensitive ECL chemiluminescence kit (Beyotime, China). This experiment was duplicated 3 times independently.

## RESULTS

### m5C regulators were differentially expressed between tumor and normal tissues in OSCC

All 21 m5C regulators were shown in Table 1, including 14 writers, four erasers and three readers. Interestingly, based on transcriptome data of OSCC extracted from TCGA, most of these regulators (17/21, 80.95%) were differentially expressed between tumor and normal tissue. Moreover, among these 17 m5C regulators, only two were down-regulated in the tumor group compared to the control group, while the remaining 15 m5C regulators were all significantly up-regulated expression in the tumor group (Figure 1), indicating that m5C modification was more active in OSCC tumor samples.

### NSUN2 was an independent risk factor for OSCC

To further assess the potential role of these 17 m5C regulators in OSCC progression, we explored their clinical prognostic value using univariate and multifactorial Cox regression analyses. The hazards ratios of these 17 m5C regulators were listed in Table S2. Surprisingly, univariate cox regression analysis showed that only NSUN2 expression was a risk factor for OSCC prognosis ($P$ <0.05) and multivariate Cox regression analysis further revealed that only NSUN2 was an independent prognostic marker for OSCC ($P$ <0.05) (Figure 2, Table S2). Therefore, NSUN2 was selected as a key gene to conduct next analysis.

### NSUN2 functioned as an immune suppressor gene

To investigate the pathway of NSUN2 in OSCC, we divided the samples into high and low expression groups according to the median value of NSUN2 expression and then performed GSEA analysis. Detailed GSEA results were listed in Table S3. For KEGG pathway enrichment analysis, natural killer cell mediated cytotoxicity, T cell receptor signaling

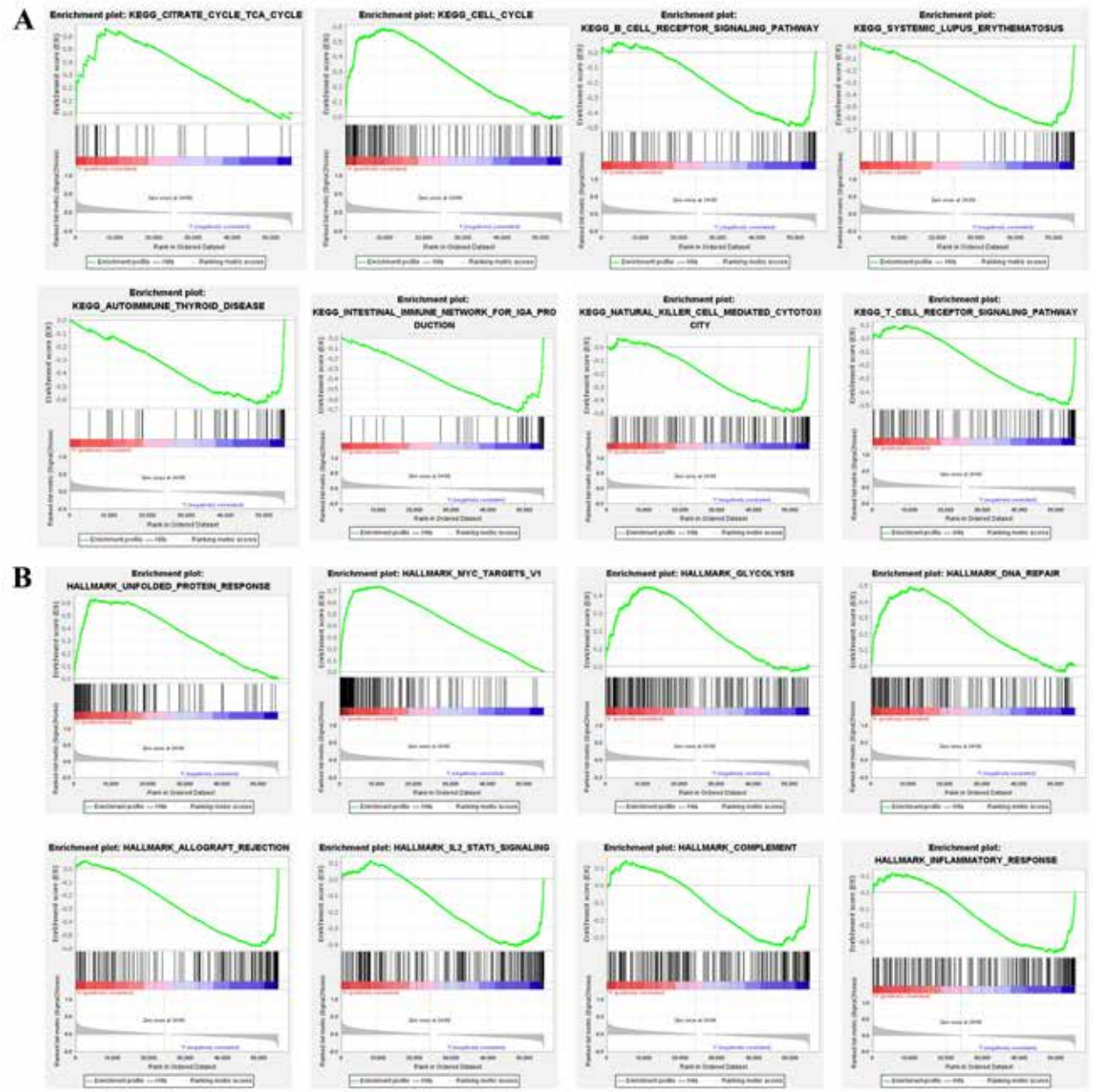


**Figure 3: NSUN2 functioned as an immune suppressor gene.** (A) The enriched KEGG pathways of NSUN2-low or high expression group identified in the GSEA. (B) The enriched Hallmark pathways of NSUN2-low or high expression group identified in the GSEA.

pathway, and B cell receptor signaling pathway and other autoimmune diseases (including systematic lupus erythematosus and autoimmune thyroid disease) were enriched in low NSUN2 group, whereas citrate cycle TCA cycle and cell cycle were enriched in high NSUN2 group (Figure 3A). Meanwhile, Hallmark pathway analysis showed that pathway-related to tumorigenesis and tumor progression were enriched in high NSUN2 group, such as MYC targets, glycolysis, and IL2-STAT3 signaling (Figure 3B). These results indicated that NSUN2 functioned as an immune suppressor gene in OSCC as pathways related to immune response were enriched in low NSUN2 group.

**NSUN2 was associated with a suppressive immune infiltration pattern**

Given that NSUN2 was associated with immune modulation by GSEA, further analysis of immune infiltration correlation was performed based on TIMER 2.0 database. Consistent with GSEA results, immune infiltration analysis further

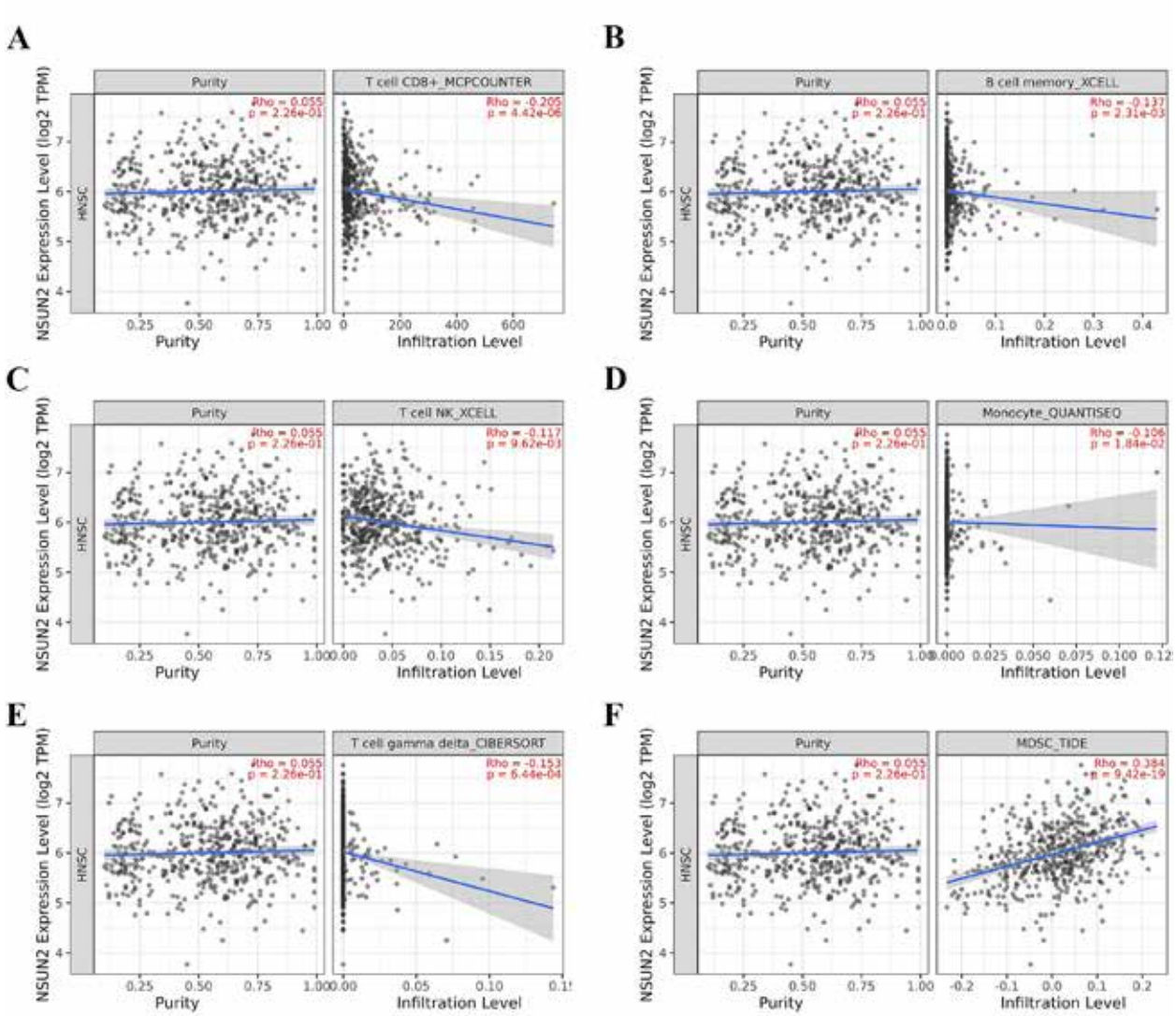


**Figure 4: NSUN2 was associated with a suppressive immune infiltration pattern.** Immune infiltration analysis by TIMER 2.0 showed that NSUN2 was negatively correlated with $CD8^+$ T cells (A), B cells (B), T and NK cells (C), memory B cells (D), gamma delta T cells (E), and positively correlated with suppressive immune cells MDSCs (F).

revealed that NSUN2 was negatively correlated with immune cells that play a dominant role in tumor killing during innate and adaptive immune response, including $CD8^+$ T cells (R = -0.205, *P* = 2.26e-01), B cells (R = -0.137, *P* = 2.31e-03), NK cells (R = -0.117, *P* = 9.62e-03), monocyte (R = -0.106, *P* = 1.84e-02), and gamma delta T cells (R = -0.153, *P* = 6.44e-04) (Figure 4A-4E). On the contrary, NSUN2 was positively correlated with myeloid-derived suppressor cells (MDSCs) (R = 0.384, *P* = 9.42e-19) which was widely known for its immune suppression function (Figure 4F). Therefore, NSUN2 was supposed to be associated with a suppressive immune infiltration pattern and thus promote tumor progression.

**NSUN2 up-regulated expression in OSCC cell lines was validated by WB**

To further validate the expression of NSUN2 *in vitro*, we examined the protein expression of NSUN2 in seven OSCC cell lines using WB. The results showed that compared with normal cells (NOK, HOK and DOK), NSUN2 expression was up-regulated in OSCC cell lines, particularly in CAL33 and SCC25 (Figure 5). This result was consistent with TCGA data mentioned in Figure 1.

## DISCUSSION

The m5C was reported to have great influence on its substrate by affecting the stability of tRNAs[19] and thus affecting translation process. In addition, m5C

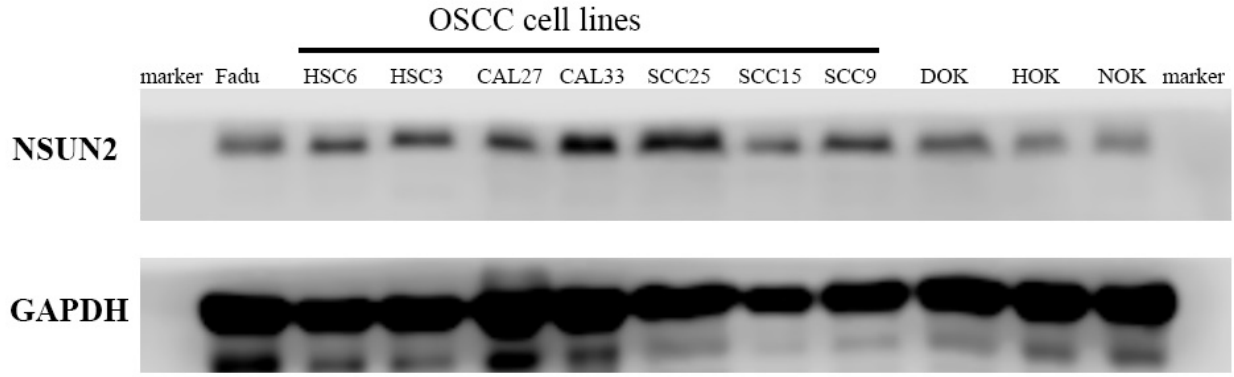


**Figure 5. NSUN2 up-regulated expression in OSCC cell lines validated by WB.** Compared with two normal oral epithelial cell lines (NOK and HOK) and DOK, NSUN2 was up-regulated in seven OSCC cell lines.

was reported to regulate mRNA export to cytoplasm, thus influencing translation[20]. Importantly, it was widely known that m5C played a pivotal role in diverse cancers by affecting tumor infiltrated immune cells. For example, as the reader of m5C, YTHDF2 enhanced anti-PD-1 immunotherapy in melanoma[21]. m5C regulators expression was associated with immune cell proportions in tumor microenvironment[22]. Similarly, the present study confirmed that most of m5C regulators (17/21) were aberrantly expressed in OSCC and that high expression of NSUN2 was an independent prognostic risk factor for OSCC, which may be conferred by NSUN2-mediated immunosuppression.

In our study, NUSN2 and many other m5C regulators were highly expressed in OSCC tumor samples, indicating that m5C modification was much more active in tumor tissues and NSUN2 was screened to be an independent marker of prognosis. In fact, as the major writer of m5C, NSUN2 was proved to participate in tumorigenesis and progression of numerous cancers. For example, in gastric cancer, NSUN2 promoted tumor proliferation through repressing $p57^{Kip2}$ in an m5C-dependent manner[23]; NSUN2 mediated methylation of H19 lncRNA was correlated to poor differentiation of hepatocellular carcinoma[24] and promoted tumor progression through m5C modification in esophageal squamous cell carcinoma[25]. Interestingly, studies have shown that NSUN2 also plays a role in HNSCC, which contains OSCC. For instance, DNA methylation mediated by NUSN2 was reported to be an independent prognostic biomarker of HNSCC[26]. These studies support our results, suggesting that NSUN2 is an oncogenic factor in OSCC.

Furthermore, the possible mechanism underlying NSUN2 prognostic value in present study was explored by GSEA and immune infiltration analysis and found that NSUN2 promoted tumor progression and affected patients' survival may be by suppressing immune reaction against tumor. Our conclusions are supported by the study by Lu *et al*, who found that NSUN2 expression level was negatively correlated with T cell activation status, and how T cell activation status influence HNSCC patients survival was dependent on NSUN2 expression level, suggesting that NSUN2 was a potential marker for immune-checkpoint blockade therapy[27]. Wang *et al* also proved that NSUN2-mediated m5C methylation negatively regulates type I interferon responses during various viral infections[28], similar results were revealed by Zhang *et al*[29]. In nasopharyngeal carcinoma, NSUN2 also promoted tumor progression through regulating immune infiltration[30]. Therefore, these studies supported that high expression of NSUN2-mediated m5C promotes OSCC progression through immunosuppression.

However, this study also has some limitations. We performed bioinformatics analysis based on TCGA samples, thereby the exact function and mechanism of NSUN2 in OSCC progression is not convincing. To address this, well designed experiments *in vitro* and *in vivo* are planned to confirm NSUN2 promoting tumor progression and its role in immune cell functions. Even so, our study provided a new prognostic marker of OSCC and indicated its function as a potential target for OSCC immune therapy.

## CONCLUSION

Most m5C regulators were highly expressed in OSCC tumor samples and NSUN2 may serve as a prognostic marker of OSCC by inhibiting immune reaction against tumor possibly.

## ACKNOWLEDGMENT

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**Conflict of interest:** The authors declare no conflicts of interest.

**Author contributions:** Xinqiong Wang collected and analyzed the data and wrote the article. Lijuan Zeng was in charge of the experimental section. Junfeng Liu designed and directed the progress of the study.
**Ethical statement:** Our study did not involve animal or human experiments, so no ethical statement is required.

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**Table S1:** Detailed clinical data

| Id | Futime | Fustat | Age | Gender | Grade | Stage | T | M | N |
|---|---|---|---|---|---|---|---|---|---|
| TCGA-CV-6433 | 641 | 0 | 57 | MALE | G3 | Stage II | T2 | unknown | N0 |
| TCGA-BA-A6DE | 440 | 0 | 70 | FEMALE | G2 | Stage II | T2 | M0 | N0 |
| TCGA-MT-A51X | 242 | 0 | 30 | MALE | G1 | Stage IVA | T1 | M0 | N2b |
| TCGA-CV-5439 | 546 | 1 | 62 | MALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-MT-A7BN | 469 | 0 | 74 | MALE | G3 | Stage IVA | unknown | unknown | unknown |
| TCGA-D6-A4Z9 | 539 | 0 | 59 | MALE | G2 | Stage IVA | T2 | M0 | N2 |
| TCGA-BA-5556 | 179 | 0 | 58 | FEMALE | G3 | Stage II | T2 | unknown | N0 |
| TCGA-MZ-A7D7 | 547 | 0 | 51 | MALE | unknown | Stage IVA | T3 | MX | N2b |
| TCGA-CN-4725 | 1157 | 0 | 60 | MALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-CV-5971 | 701 | 0 | 60 | MALE | G2 | Stage IVA | T4a | unknown | N2a |
| TCGA-CV-A45T | 4856 | 1 | 64 | FEMALE | G3 | unknown | T1 | M0 | N0 |
| TCGA-CR-6472 | 1050 | 0 | 59 | MALE | G3 | unknown | unknown | unknown | unknown |
| TCGA-CR-7401 | 1077 | 0 | 64 | MALE | G2 | Stage I | T1 | M0 | N0 |
| TCGA-HD-8634 | 385 | 1 | 51 | FEMALE | G2 | Stage I | T1 | MX | N0 |
| TCGA-CQ-A4CB | 893 | 0 | 59 | MALE | G2 | Stage III | T1 | M0 | N1 |
| TCGA-CV-7255 | 64 | 1 | 32 | FEMALE | G2 | Stage IVA | T4a | unknown | N0 |
| TCGA-CN-6995 | 112 | 1 | 78 | MALE | G2 | Stage IVA | T4a | unknown | N0 |
| TCGA-CN-6996 | 530 | 1 | 58 | FEMALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-CQ-A4CE | 785 | 0 | 76 | FEMALE | G2 | Stage II | T2 | M0 | N0 |
| TCGA-QK-A6II | 284 | 1 | 52 | MALE | G2 | Stage IVA | T4a | M0 | N2c |
| TCGA-CR-7390 | 1508 | 0 | 67 | MALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-BA-6871 | 63 | 0 | 75 | MALE | G2 | unknown | TX | unknown | NX |
| TCGA-CX-7086 | 573 | 0 | 53 | MALE | G2 | Stage III | T3 | unknown | N1 |
| TCGA-CN-4733 | 1586 | 0 | 61 | MALE | G3 | Stage III | T1 | unknown | N1 |
| TCGA-CV-A6JU | 110 | 0 | 61 | FEMALE | G2 | Stage IVB | T4b | M0 | N0 |
| TCGA-CV-7236 | 144 | 1 | 77 | FEMALE | G3 | Stage IVA | T3 | unknown | N2c |
| TCGA-BB-7871 | 750 | 0 | 64 | FEMALE | G2 | Stage IVA | T4a | unknown | N2c |
| TCGA-IQ-A61K | 161 | 1 | 70 | FEMALE | G2 | Stage IVA | T3 | M0 | N2b |
| TCGA-QK-A6VB | 450 | 0 | 66 | MALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-WA-A7H4 | 443 | 0 | 69 | MALE | G3 | Stage II | T2 | unknown | N0 |
| TCGA-BB-7863 | 1025 | 0 | 43 | FEMALE | G2 | Stage III | T3 | unknown | N0 |
| TCGA-4P-AA8J | 102 | 0 | 66 | MALE | G2 | Stage IVA | T2 | MX | N2c |
| TCGA-UF-A7JS | 680 | 1 | 59 | MALE | G2 | Stage IVA | T4a | M0 | N2b |
| TCGA-DQ-5630 | 1030 | 0 | 73 | MALE | G2 | unknown | TX | unknown | NX |
| TCGA-CV-5976 | 1478 | 0 | 50 | MALE | G2 | Stage IVA | T4a | unknown | N2b |
| TCGA-CX-7085 | 321 | 0 | 77 | FEMALE | G2 | Stage I | T1 | unknown | N0 |
| TCGA-D6-6823 | 701 | 0 | 50 | MALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-CV-5436 | 584 | 1 | 65 | MALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-CV-6948 | 1289 | 1 | 79 | FEMALE | G2 | Stage IVB | T4a | unknown | N3 |
| TCGA-P3-A5QE | 1559 | 0 | 49 | MALE | G2 | Stage III | T2 | MX | N1 |
| TCGA-BA-5149 | 248 | 0 | 47 | MALE | G2 | Stage IVA | T3 | unknown | N2c |
| TCGA-HD-7831 | 361 | 0 | 74 | MALE | G2 | Stage IVA | T2 | unknown | N2 |
| TCGA-CV-7235 | 2347 | 0 | 67 | MALE | G3 | Stage II | T2 | unknown | N0 |
| TCGA-T2-A6WZ | 484 | 1 | 53 | MALE | G2 | unknown | T3 | unknown | N2b |
| TCGA-UF-A71E | 1278 | 0 | 63 | MALE | G3 | Stage IVA | T4a | M0 | N0 |
| TCGA-UP-A6WW | 518 | 0 | 58 | MALE | G2 | unknown | unknown | unknown | unknown |
| TCGA-UF-A719 | 1663 | 0 | 54 | MALE | G1 | Stage II | T2 | M0 | N0 |
| TCGA-DQ-7592 | 516 | 0 | 57 | MALE | G2 | unknown | TX | unknown | NX |
| TCGA-CV-6951 | 915 | 1 | 57 | MALE | G2 | Stage IVA | T4a | unknown | N2c |
| TCGA-CN-A6V6 | 234 | 0 | 59 | MALE | GX | Stage IVA | T2 | MX | N2b |
| TCGA-CV-5973 | 2641 | 0 | 62 | FEMALE | G3 | Stage III | T3 | unknown | N1 |
| TCGA-CV-6961 | 76 | 1 | 61 | MALE | G3 | Stage II | T2 | unknown | N0 |
| TCGA-F7-A61S | 576 | 0 | 62 | MALE | G1 | Stage III | T3 | M0 | N0 |
| TCGA-CV-A45P | 639 | 0 | 82 | FEMALE | G2 | Stage I | T1 | M0 | N0 |
| TCGA-CN-4742 | 397 | 1 | 48 | FEMALE | G3 | Stage IVA | T4a | M0 | N2b |
| TCGA-KU-A6H8 | 327 | 1 | 41 | MALE | G3 | Stage IVA | T2 | M0 | N2b |
| TCGA-CN-5364 | 493 | 1 | 55 | MALE | G2 | Stage IVA | T4a | M0 | N2c |
| TCGA-CV-7102 | 56 | 1 | 76 | FEMALE | G3 | Stage IVA | T3 | unknown | N2 |
| TCGA-CV-A463 | 23 | 1 | 82 | FEMALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-CV-7103 | 1591 | 1 | 49 | MALE | G2 | Stage IVA | T2 | unknown | N2b |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TCGA-CN-A642 | 82 | 1 | 57 | MALE | G3 | Stage IVB | T4a | M0 | N3 |
| TCGA-CV-6943 | 602 | 1 | 74 | MALE | G2 | Stage III | T3 | unknown | NX |
| TCGA-HD-8635 | 695 | 0 | 61 | FEMALE | G2 | Stage III | T1 | MX | N1 |
| TCGA-QK-AA3K | 253 | 0 | 60 | MALE | G2 | Stage IVA | T3 | MX | N2b |
| TCGA-CR-5250 | 799 | 0 | 71 | MALE | G3 | unknown | TX | unknown | NX |
| TCGA-QK-A8Z9 | 352 | 0 | 56 | MALE | G2 | Stage IVA | T4a | M0 | N2b |
| TCGA-F7-A620 | 543 | 0 | 47 | MALE | G1 | Stage III | T3 | M0 | N1 |
| TCGA-CV-6952 | 185 | 1 | 65 | FEMALE | G1 | Stage IVA | T3 | unknown | N2b |
| TCGA-CN-A6UY | 307 | 0 | 57 | MALE | G2 | Stage IVA | T3 | MX | N2b |
| TCGA-WA-A7GZ | 625 | 1 | 58 | MALE | G2 | unknown | T2 | unknown | N0 |
| TCGA-C9-A480 | 386 | 0 | 45 | FEMALE | G1 | Stage III | T3 | M0 | N0 |
| TCGA-IQ-A6SH | 471 | 0 | 55 | MALE | G1 | Stage III | T2 | M0 | N1 |
| TCGA-CR-6477 | 514 | 0 | 56 | FEMALE | G2 | unknown | unknown | unknown | unknown |
| TCGA-IQ-A61L | 416 | 0 | 72 | FEMALE | G1 | Stage II | T2 | M0 | N0 |
| TCGA-CV-7238 | 2727 | 0 | 69 | FEMALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-CQ-A4C9 | 707 | 0 | 56 | MALE | G2 | Stage III | T2 | M0 | N1 |
| TCGA-CQ-6219 | 479 | 1 | 50 | FEMALE | G2 | Stage IVA | T3 | unknown | N2a |
| TCGA-CR-6488 | 379 | 0 | 68 | FEMALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-HD-8224 | 446 | 1 | 63 | MALE | G3 | Stage IVA | T3 | M0 | N2c |
| TCGA-CQ-5333 | 341 | 1 | 74 | MALE | G3 | Stage II | T2 | unknown | N0 |
| TCGA-D6-8569 | 770 | 0 | 52 | MALE | G2 | Stage II | T2 | M0 | N0 |
| TCGA-C9-A47Z | 191 | 1 | 72 | FEMALE | G1 | Stage III | T2 | M0 | N1 |
| TCGA-F7-A61V | 384 | 0 | 54 | MALE | G1 | Stage II | T2 | M0 | N0 |
| TCGA-CV-6934 | 65 | 1 | 66 | FEMALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-F7-8489 | 658 | 0 | 48 | MALE | G1 | Stage II | T2 | M0 | N0 |
| TCGA-F7-A61W | 14 | 0 | 51 | MALE | G2 | Stage IVA | T2 | M0 | N2b |
| TCGA-CV-A465 | 215 | 1 | 24 | MALE | G1 | Stage III | T3 | M0 | N0 |
| TCGA-CQ-6222 | 1407 | 0 | 63 | MALE | G2 | Stage IVA | T2 | unknown | N2b |
| TCGA-IQ-A61J | 1021 | 0 | 54 | MALE | G1 | Stage IVA | T2 | M0 | N2b |
| TCGA-CV-6954 | 2002 | 1 | 59 | MALE | G2 | Stage IVA | T4a | unknown | NX |
| TCGA-CN-6019 | 432 | 0 | 61 | MALE | G2 | Stage IVA | T4a | unknown | N0 |
| TCGA-P3-A5QA | 1726 | 0 | 41 | MALE | G3 | Stage I | T1 | MX | NX |
| TCGA-CV-7446 | 1093 | 1 | 66 | MALE | G2 | Stage IVA | T2 | unknown | N2b |
| TCGA-CR-7393 | 993 | 0 | 26 | MALE | G2 | Stage III | T1 | M0 | N1 |
| TCGA-CN-6017 | 629 | 0 | 55 | MALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-P3-A6T0 | 578 | 0 | 47 | FEMALE | G2 | Stage IVA | T4a | MX | N0 |
| TCGA-CN-A640 | 134 | 1 | 40 | FEMALE | G2 | Stage IVA | T2 | MX | N2b |
| TCGA-DQ-5625 | 1133 | 1 | 52 | FEMALE | G2 | unknown | TX | unknown | NX |
| TCGA-CQ-7065 | 1335 | 0 | 40 | MALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-CV-A6K0 | 606 | 0 | 58 | MALE | G3 | Stage I | T1 | M0 | N0 |
| TCGA-CN-5359 | 377 | 1 | 59 | MALE | G2 | Stage IVA | T4a | M0 | N2b |
| TCGA-BB-7861 | 682 | 0 | 56 | MALE | unknown | Stage III | T1 | unknown | N1 |
| TCGA-H7-A6C4 | 414 | 0 | 35 | FEMALE | G2 | Stage IVA | T2 | M0 | N2b |
| TCGA-CQ-6218 | 1253 | 0 | 52 | FEMALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-UF-A71A | 86 | 1 | 67 | MALE | G1 | Stage IVA | T4a | M0 | N2c |
| TCGA-T2-A6WX | 209 | 1 | 73 | FEMALE | G1 | unknown | unknown | unknown | unknown |
| TCGA-P3-A6T7 | 487 | 1 | 55 | MALE | G2 | Stage IVA | T3 | MX | N2b |
| TCGA-CX-A4AQ | 1555 | 0 | 56 | MALE | G3 | Stage IVA | T2 | M0 | N2b |
| TCGA-CV-6950 | 459 | 1 | 64 | MALE | G2 | Stage IVA | T3 | unknown | N2c |
| TCGA-IQ-A6SG | 579 | 0 | 61 | FEMALE | G2 | Stage III | T3 | M0 | N0 |
| TCGA-DQ-7593 | 369 | 0 | 58 | MALE | G4 | unknown | TX | unknown | NX |
| TCGA-CQ-A4C7 | 353 | 1 | 88 | MALE | G3 | Stage III | T3 | M0 | N1 |
| TCGA-MT-A67A | 914 | 0 | 85 | FEMALE | G2 | Stage I | T1 | MX | N0 |
| TCGA-CV-A45R | 5480 | 0 | 46 | MALE | G1 | Stage III | T2 | M0 | N1 |
| TCGA-CR-7397 | 754 | 0 | 44 | MALE | G2 | Stage IVA | T3 | M0 | N2b |
| TCGA-CV-6441 | 292 | 1 | 60 | MALE | G3 | Stage III | T3 | unknown | N0 |
| TCGA-CQ-6224 | 1350 | 0 | 52 | MALE | G3 | Stage IVA | T2 | unknown | N2b |
| TCGA-CV-5970 | 406 | 1 | 59 | MALE | G2 | Stage IVA | T4a | unknown | N2b |
| TCGA-CV-7180 | 327 | 1 | 34 | MALE | G2 | Stage II | T2 | unknown | NX |
| TCGA-BA-6873 | 122 | 0 | 28 | MALE | G2 | Stage IVA | T4a | unknown | N2b |
| TCGA-CV-7438 | 194 | 1 | 87 | FEMALE | G3 | Stage I | T1 | unknown | NX |
| TCGA-CV-A6JO | 197 | 1 | 69 | MALE | G2 | Stage IVA | T3 | M0 | N2c |
| TCGA-CN-6998 | 46 | 0 | 53 | MALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-BA-A6D8 | 850 | 0 | 59 | MALE | G2 | Stage IVA | T4a | M0 | N2c |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TCGA-CX-7219 | 1045 | 0 | 47 | MALE | G2 | Stage IVA | T4a | unknown | N2c |
| TCGA-CV-7243 | 954 | 0 | 50 | MALE | G2 | Stage III | T2 | unknown | N1 |
| TCGA-CR-7391 | 913 | 0 | 36 | FEMALE | G1 | Stage I | T1 | M0 | N0 |
| TCGA-QK-A8Z7 | 198 | 0 | 59 | MALE | unknown | Stage IVA | T4a | M0 | N2c |
| TCGA-HD-A6HZ | 111 | 0 | 79 | FEMALE | G2 | Stage III | T2 | MX | N1 |
| TCGA-CQ-6221 | 1000 | 0 | 79 | MALE | G3 | unknown | T2 | unknown | NX |
| TCGA-DQ-5624 | 1778 | 0 | 43 | FEMALE | G2 | unknown | TX | unknown | NX |
| TCGA-CN-A498 | 443 | 0 | 61 | FEMALE | G1 | Stage III | T3 | MX | N0 |
| TCGA-CR-6493 | 282 | 1 | 69 | MALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-CR-6491 | 693 | 0 | 60 | MALE | G2 | Stage IVA | T4a | M0 | N2b |
| TCGA-CV-A45X | 198 | 1 | 47 | MALE | G2 | Stage IVA | T2 | M0 | N2b |
| TCGA-CN-4736 | 395 | 1 | 70 | FEMALE | G2 | unknown | T1 | unknown | NX |
| TCGA-CQ-7072 | 1950 | 0 | 51 | MALE | G3 | Stage II | T2 | M0 | N0 |
| TCGA-IQ-A61E | 1147 | 0 | 55 | FEMALE | G2 | Stage III | T3 | MX | N0 |
| TCGA-BA-A6DG | 69 | 1 | 49 | MALE | G2 | unknown | TX | MX | NX |
| TCGA-QK-A6IJ | 387 | 0 | 71 | MALE | G3 | Stage III | T2 | M0 | N1 |
| TCGA-CV-A6JT | 852 | 0 | 65 | MALE | G2 | Stage II | T2 | M0 | N0 |
| TCGA-CQ-A4CH | 371 | 0 | 58 | MALE | G2 | Stage II | T2 | M0 | N0 |
| TCGA-BA-A6DB | 216 | 0 | 24 | FEMALE | G1 | Stage I | T1 | M0 | N0 |
| TCGA-CR-7372 | 759 | 0 | 45 | MALE | G1 | Stage I | T1 | M0 | N0 |
| TCGA-CQ-5327 | 1660 | 0 | 61 | FEMALE | G2 | Stage IVA | T3 | unknown | N2c |
| TCGA-D6-6825 | 491 | 0 | 73 | MALE | G2 | Stage I | T1 | unknown | N0 |
| TCGA-BA-6872 | 384 | 1 | 47 | MALE | G2 | unknown | TX | unknown | NX |
| TCGA-HD-8314 | 670 | 0 | 58 | MALE | G4 | Stage III | T1 | unknown | N1 |
| TCGA-CQ-5330 | 1393 | 0 | 69 | FEMALE | G3 | Stage IVA | T3 | unknown | N2b |
| TCGA-QK-A652 | 357 | 0 | 60 | MALE | G2 | Stage III | T1 | MX | N1 |
| TCGA-BB-8601 | 624 | 0 | 84 | MALE | G2 | Stage III | T3 | MX | N1 |
| TCGA-CN-4737 | 625 | 0 | 19 | MALE | G2 | Stage IVA | T2 | unknown | N2b |
| TCGA-CV-6436 | 1899 | 0 | 62 | MALE | G1 | Stage IVA | T4a | unknown | N0 |
| TCGA-BA-7269 | 1273 | 0 | 61 | MALE | G1 | Stage III | T2 | unknown | N1 |
| TCGA-CN-5373 | 1584 | 0 | 55 | FEMALE | G1 | Stage I | T1 | unknown | N0 |
| TCGA-D6-A4ZB | 376 | 0 | 61 | MALE | G2 | Stage III | T3 | M0 | N0 |
| TCGA-BA-4075 | 283 | 1 | 49 | MALE | G2 | Stage III | T3 | M0 | N0 |
| TCGA-CV-6939 | 666 | 1 | 60 | MALE | G3 | Stage IVA | T4a | unknown | N2b |
| TCGA-MT-A67D | 56 | 0 | 55 | MALE | G2 | Stage II | T2 | M0 | N0 |
| TCGA-HD-7917 | 342 | 0 | 62 | MALE | G1 | Stage II | T2 | unknown | N0 |
| TCGA-D6-A6EO | 435 | 0 | 44 | MALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-CR-7382 | 796 | 0 | 49 | MALE | G2 | Stage IVA | T2 | M0 | N2c |
| TCGA-CV-6936 | 166 | 1 | 68 | MALE | G2 | Stage IVA | T4a | unknown | N2c |
| TCGA-CV-7407 | 1081 | 1 | 67 | FEMALE | G2 | Stage II | T2 | unknown | NX |
| TCGA-DQ-5631 | 548 | 1 | 52 | MALE | G3 | unknown | TX | unknown | NX |
| TCGA-BA-A6DD | 173 | 1 | 44 | MALE | G2 | Stage IVA | T4a | M0 | N2c |
| TCGA-IQ-A61G | 360 | 0 | 57 | MALE | G2 | Stage IVA | T4a | MX | N2c |
| TCGA-DQ-7591 | 622 | 0 | 62 | MALE | G4 | unknown | TX | unknown | NX |
| TCGA-CQ-6229 | 1179 | 0 | 61 | MALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-BA-A6DF | 238 | 1 | 80 | FEMALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-CR-7394 | 1346 | 0 | 70 | MALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-P3-A6T8 | 400 | 0 | 54 | MALE | G3 | Stage IVA | T4a | MX | N2 |
| TCGA-IQ-A61H | 1138 | 0 | 76 | MALE | G2 | Stage II | T2 | MX | N0 |
| TCGA-CQ-5329 | 1422 | 0 | 46 | FEMALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-D6-6515 | 403 | 1 | 82 | FEMALE | G3 | Stage II | T2 | unknown | N0 |
| TCGA-UF-A7JO | 631 | 1 | 79 | FEMALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-CN-6020 | 205 | 1 | 58 | MALE | G2 | Stage III | T2 | unknown | N1 |
| TCGA-CV-7406 | 1748 | 1 | 49 | MALE | GX | Stage II | T2 | unknown | N0 |
| TCGA-CV-6945 | 366 | 1 | 41 | MALE | G2 | Stage IVA | T4a | unknown | N2 |
| TCGA-BA-4077 | 1134 | 1 | 45 | FEMALE | G2 | Stage IVA | T4a | M0 | N0 |
| TCGA-CN-5358 | 261 | 1 | 60 | MALE | G2 | Stage II | T2 | unknown | N0 |
| TCGA-KU-A66T | 552 | 0 | 53 | FEMALE | G2 | Stage IVA | T4 | MX | N0 |
| TCGA-T3-A92N | 95 | 1 | 79 | MALE | G3 | Stage IVA | T2 | MX | N2c |
| TCGA-HD-7832 | 350 | 0 | 52 | MALE | G2 | Stage IVA | T4a | unknown | N0 |
| TCGA-F7-A50G | 616 | 0 | 66 | MALE | G1 | Stage III | T3 | M0 | N1 |
| TCGA-CR-7392 | 1425 | 0 | 67 | FEMALE | G1 | Stage IVA | T3 | M0 | N2b |
| TCGA-CV-6953 | 1641 | 1 | 80 | FEMALE | G1 | Stage III | T3 | unknown | N0 |
| TCGA-P3-A6T4 | 62 | 1 | 54 | MALE | G2 | Stage IVA | T4a | MX | N1 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| TCGA-CV-6003 | 1665 | 0 | 50 | FEMALE | G2 | Stage III | T2 | unknown | N1 |
| TCGA-CV-5979 | 1315 | 0 | 26 | MALE | G2 | Stage IVA | T2 | unknown | N2b |
| TCGA-BB-4224 | 278 | 0 | 52 | MALE | G2 | Stage IVA | T2 | unknown | N2b |
| TCGA-DQ-7594 | 489 | 0 | 47 | MALE | G4 | unknown | TX | unknown | NX |
| TCGA-CV-6941 | 342 | 1 | 51 | MALE | G2 | Stage III | T3 | unknown | N0 |
| TCGA-CN-6024 | 224 | 0 | 66 | MALE | G2 | Stage IVA | T4a | M0 | N2c |
| TCGA-CN-6016 | 594 | 0 | 64 | MALE | G2 | Stage IVA | T4a | unknown | N1 |
| TCGA-BB-A6UO | 268 | 1 | 61 | FEMALE | G2 | Stage IVA | T4a | MX | N2b |
| TCGA-CQ-5325 | 654 | 1 | 65 | MALE | G2 | Stage I | T1 | unknown | N0 |
| TCGA-BB-4225 | 146 | 0 | 73 | MALE | G3 | unknown | TX | unknown | NX |
| TCGA-CV-A6JD | 182 | 1 | 82 | FEMALE | G3 | unknown | unknown | unknown | unknown |
| TCGA-CQ-A4CA | | 0 | unknown | MALE | G2 | Stage II | T2 | M0 | N0 |
| TCGA-CV-6959 | 256 | 1 | 48 | MALE | G2 | Stage III | T3 | unknown | N0 |
| TCGA-UF-A7JT | 993 | 1 | 72 | FEMALE | G3 | Stage IVA | T4a | M0 | N0 |
| TCGA-F7-A50J | 585 | 0 | 67 | FEMALE | G2 | Stage III | T3 | M0 | N0 |
| TCGA-CN-5370 | 259 | 1 | 78 | MALE | G3 | Stage III | T3 | M0 | N1 |
| TCGA-CV-7104 | 393 | 1 | 61 | FEMALE | G2 | Stage IVA | T2 | unknown | N2b |
| TCGA-UF-A7JC | 546 | 1 | 42 | MALE | G1 | Stage IVA | T3 | M0 | N2b |
| TCGA-CN-5367 | 352 | 1 | 60 | FEMALE | G2 | Stage IVA | T4a | M0 | N2b |
| TCGA-CV-6933 | 2741 | 1 | 53 | MALE | G2 | Stage III | T3 | unknown | N1 |
| TCGA-BA-4074 | 462 | 1 | 69 | MALE | G3 | Stage IVA | T2 | M0 | N2c |
| TCGA-BB-4228 | 558 | 0 | 50 | MALE | GX | Stage III | T3 | unknown | NX |
| TCGA-CV-5977 | 1840 | 0 | 66 | MALE | G2 | Stage IVA | T3 | unknown | N2b |
| TCGA-CV-6956 | 217 | 1 | 67 | MALE | G2 | Stage III | T3 | unknown | N1 |

**Table S2:** Prognostic value of m5C regulators' by cox analysis

| Gene names | Univariate cox | | Multivariate cox | |
|---|---|---|---|---|
| | HR | *P* value | HR | *P* value |
| DNMT1 | 0.994 | 0.797 | 0.990 | 0.675 |
| DNMT2 | 0.994 | 0.797 | 0.990 | 0.675 |
| DNMT3A | 0.880 | 0.219 | 0.818 | 0.090 |
| DNMT3B | 0.952 | 0.423 | 0.957 | 0.502 |
| NSUN | 0.943 | 0.839 | 0.799 | 0.415 |
| NSUN2 | 1.037 | 0.024 | 1.045 | 0.013 |
| NUSN3 | 1.166 | 0.341 | 1.041 | 0.801 |
| NUSN4 | 1.160 | 0.328 | 1.147 | 0.424 |
| NSUN5 | 1.022 | 0.408 | 0.995 | 0.866 |
| NSUN7 | 0.943 | 0.839 | 0.799 | 0.415 |
| TRM4A | 1.009 | 0.860 | 1.037 | 0.580 |
| TRM4B | 1.009 | 0.860 | 1.037 | 0.580 |
| TET3 | 0.997 | 0.945 | 1.004 | 0.930 |
| ALKBH1 | 1.009 | 0.860 | 1.037 | 0.580 |
| ALYREF | 0.996 | 0.481 | 0.994 | 0.221 |
| YTHDF2 | 1.046 | 0.053 | 1.048 | 0.055 |
| YBX1 | 1.000 | 0.837 | 0.999 | 0.598 |

**Table S3:** Pathways enriched by GSEA based on NSUN2 expression level

| | NAME | SIZE | NES | NOM p-val | FDR q-val |
|---|---|---|---|---|---|
| Gene set enriched in NSUN2 high expression group (KEGG) | KEGG_BASAL_TRANSCRIPTION_FACTORS | 35 | 2.00264 | 0 | 0.06574205 |
| | KEGG_SPLICEOSOME | 127 | 1.9582235 | 0 | 0.05904996 |
| | KEGG_RNA_DEGRADATION | 59 | 1.92475 | 0 | 0.04734504 |
| | KEGG_UBIQUITIN_MEDIATED_PROTEOLYSIS | 134 | 1.9331985 | 0.001949318 | 0.060278896 |
| | KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ ANCHOR_BIOSYNTHESIS | 25 | 1.8189408 | 0.001956947 | 0.074131906 |
| | KEGG_HOMOLOGOUS_RECOMBINATION | 28 | 1.74577 | 0.001980198 | 0.09491764 |
| | KEGG_NUCLEOTIDE_EXCISION_REPAIR | 44 | 1.8637778 | 0.001984127 | 0.061065078 |
| | KEGG_AMINOACYL_TRNA_BIOSYNTHESIS | 22 | 1.8991205 | 0.001996008 | 0.050276775 |
| | KEGG_PROTEIN_EXPORT | 24 | 1.8587042 | 0.00952381 | 0.0551498 |
| | KEGG_CELL_CYCLE | 124 | 1.7970127 | 0.00984252 | 0.07905578 |
| | KEGG_PYRIMIDINE_METABOLISM | 97 | 1.6757734 | 0.013944224 | 0.121652275 |
| | KEGG_PENTOSE_AND_GLUCURONATE_ INTERCONVERSIONS | 28 | 1.6694181 | 0.014028057 | 0.11364856 |
| | KEGG_AMINO_SUGAR_AND_NUCLEOTIDE_SUGAR_ METABOLISM | 42 | 1.6865331 | 0.016423358 | 0.12756246 |
| | KEGG_CITRATE_CYCLE_TCA_CYCLE | 31 | 1.7539074 | 0.020833334 | 0.09452936 |
| | KEGG_OOCYTE_MEIOSIS | 112 | 1.6215094 | 0.021868788 | 0.1442419 |
| | KEGG_STEROID_BIOSYNTHESIS | 17 | 1.7686858 | 0.02244898 | 0.091677286 |
| | KEGG_PROTEASOME | 46 | 1.6722208 | 0.023391813 | 0.11825072 |
| | KEGG_ONE_CARBON_POOL_BY_FOLATE | 17 | 1.6984322 | 0.023715414 | 0.12426693 |
| | KEGG_ALANINE_ASPARTATE_AND_GLUTAMATE_ METABOLISM | 30 | 1.6202043 | 0.025 | 0.13833348 |
| | KEGG_TERPENOID_BACKBONE_BIOSYNTHESIS | 15 | 1.6853509 | 0.030364372 | 0.12014868 |
| | KEGG_RNA_POLYMERASE | 28 | 1.6480917 | 0.032629557 | 0.12564254 |
| | KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION | 56 | 1.5362582 | 0.046332046 | 0.22594178 |
| | KEGG_SNARE_INTERACTIONS_IN_VESICULAR_ TRANSPORT | 38 | 1.5170963 | 0.046511628 | 0.22173798 |
| | KEGG_PORPHYRIN_AND_CHLOROPHYLL_METABOLISM | 40 | 1.4956746 | 0.04914934 | 0.2311612 |
| Gene set enriched in NSUN2 low expression group (KEGG) | KEGG_CELL_ADHESION_MOLECULES_CAMS | 131 | -1.9137322 | 0.006147541 | 0.1403281 |
| | KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_ PRODUCTION | 46 | -1.8519346 | 0.008130081 | 0.13651742 |
| | KEGG_ASTHMA | 28 | -1.8243718 | 0.016260162 | 0.119587906 |
| | KEGG_PRIMARY_ IMMUNODEFICIENCY | 35 | -1.7834059 | 0.017374517 | 0.1292005 |
| | KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS | 55 | -1.7640917 | 0.030612245 | 0.10288887 |
| | KEGG_HEMATOPOIETIC_CELL_LINEAGE | 85 | -1.7756453 | 0.031746034 | 0.1094177 |
| | KEGG_NATURAL_KILLER_CELL_MEDIATED_ CYTOTOXICITY | 132 | -1.6712017 | 0.032 | 0.16008481 |
| | KEGG_ALDOSTERONE_REGULATED_SODIUM_ REABSORPTION | 42 | -1.536611 | 0.034 | 0.20558833 |
| | KEGG_CYTOKINE_CYTOKINE_RECEPTOR_INTERACTION | 264 | -1.6669477 | 0.034623217 | 0.14641449 |
| | KEGG_ALLOGRAFT_REJECTION | 35 | -1.6641535 | 0.041152265 | 0.134471 |
| | KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY | 108 | -1.6110556 | 0.04233871 | 0.16274302 |
| | KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY | 75 | -1.5612998 | 0.04733728 | 0.2054608 |
| | KEGG_AUTOIMMUNE_THYROID_DISEASE | 50 | -1.7026874 | 0.048387095 | 0.14349282 |
| | KEGG_CELL_ADHESION_MOLECULES_CAMS | 131 | -1.9137322 | 0.006147541 | 0.1403281 |
| | KEGG_INTESTINAL_IMMUNE_NETWORK_FOR_IGA_ PRODUCTION | 46 | -1.8519346 | 0.008130081 | 0.13651742 |
| | KEGG_ASTHMA | 28 | -1.8243718 | 0.016260162 | 0.119587906 |
| | KEGG_PRIMARY_IMMUNODEFICIENCY | 35 | -1.7834059 | 0.017374517 | 0.1292005 |
| | KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS | 55 | -1.7640917 | 0.030612245 | 0.10288887 |
| | KEGG_HEMATOPOIETIC_CELL_LINEAGE | 85 | -1.7756453 | 0.031746034 | 0.1094177 |
| | KEGG_NATURAL_KILLER_CELL_MEDIATED_ CYTOTOXICITY | 132 | -1.6712017 | 0.032 | 0.16008481 |

| | | | | | |
|---|---|---|---|---|---|
| Gene set enriched in NSUN2 high expression group (Hallmarker) | HALLMARK_UNFOLDED_PROTEIN_RESPONSE | 110 | 2.1890829 | 0 | 0.001614922 |
| | HALLMARK_PROTEIN_ SECRETION | 96 | 2.097992 | 0 | 0.003388631 |
| | HALLMARK_MYC_TARGETS_V1 | 196 | 2.0664613 | 0 | 0.003699104 |
| | HALLMARK_MTORC1_ SIGNALING | 197 | 2.0237246 | 0 | 0.004903375 |
| | HALLMARK_G2M_CHECKPOINT | 196 | 1.9573319 | 0.00204918 | 0.008849388 |
| | HALLMARK_MYC_TARGETS_V2 | 58 | 1.8883637 | 0.002169197 | 0.01656526 |
| | HALLMARK_GLYCOLYSIS | 198 | 1.7320337 | 0.008179959 | 0.05666993 |
| | HALLMARK_E2F_TARGETS | 198 | 1.7801405 | 0.012195122 | 0.044069212 |
| | HALLMARK_WNT_BETA_CATENIN_SIGNALING | 42 | 1.5411913 | 0.039014373 | 0.1347896 |
| | HALLMARK_ANDROGEN_RESPONSE | 99 | 1.5049654 | 0.043209877 | 0.14770037 |
| | HALLMARK_DNA_REPAIR | 149 | 1.6088133 | 0.046709128 | 0.103049584 |
| Gene set enriched in NSUN2 low expression group (Hallmarker) | HALLMARK_ALLOGRAFT_REJECTION | 196 | -1.7241435 | 0.039447732 | 0.2925684 |
| | HALLMARK_IL2_STAT5_ SIGNALING | 199 | -1.4903085 | 0.068627454 | 0.5693784 |
| | HALLMARK_APICAL_SURFACE | 44 | -1.3597964 | 0.106177606 | 0.5270397 |
| | HALLMARK_KRAS_SIGNALING_DN | 199 | -1.253931 | 0.13465346 | 0.6405929 |
| | HALLMARK_COAGULATION | 138 | -1.2388859 | 0.1826923 | 0.48970753 |
| | HALLMARK_COMPLEMENT | 200 | -1.2334889 | 0.20116054 | 0.43777278 |
| | HALLMARK_MYOGENESIS | 199 | -1.3629454 | 0.22287968 | 0.69589704 |
| | HALLMARK_KRAS_SIGNALING_UP | 199 | -1.2397141 | 0.23658052 | 0.5702866 |
| | HALLMARK_INFLAMMATORY_RESPONSE | 200 | -1.1877587 | 0.26923078 | 0.45403984 |
| | HALLMARK_IL6_JAK_STAT3_ SIGNALING | 87 | -1.1527238 | 0.3 | 0.4592749 |
| | HALLMARK_INTERFERON_GAMMA_RESPONSE | 198 | -1.0127723 | 0.46679688 | 0.6525441 |

Original Article

# Serum Netrin-1 as a biomarker for ovarian cancer detection

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## ABSTRACT

**Objective:** This study aimed to determine the value of netrin-1 in epithelial ovarian cancer (EOC), which has high morbidity and mortality.
**Design**: This research is a cross-sectional type descriptive study.
**Setting:** The study was carried out at the Gynecological Oncology Department of Sakarya University Training and Research Hospital.
**Subjects:** Female patients with a diagnosis of adnexal mass
**Interventions:** Serum netrin 1 levels of patients with benign (n=43) and malignant (n=40) histopathology operated in our clinic with the diagnosis of suspected adnexal mass were evaluated. The netrin 1 levels of both groups were compared and the receiver operating characteristic analysis was carried out.
**Main outcome measures:** Serum netrin-1 levels in patients with a diagnosis of ovarian cancer.
**Results:** While netrin-1 level was found to be higher in patients with EOC, the cut-off value was found to be 325 pg/mL. AUC=0.690, p=0.003 (95%CI:0.571-0.809) was determined to be able to differentiate between malignant and benign adnexal masses by means of the netrin 1 level. The sensitivity of the netrin-1 level for the diagnosis of EOC is 60%, and the specificity is 83.7%.
**Conclusion:** Serum netrin-1 may be a potential biomarker in patients with EOC.

**KEY WORDS:** apoptosis, biomarker, epithelial ovarian cancer, Netrin-1, ovarian cancer diagnosis

## INTRODUCTION

Although ovarian cancer is the second most common malignancy cause in women after endometrial cancer, it ranks first in deaths due to gynecological malignancies[1]. The 5-year survival rate in patients remains below 30% because the disease is asymptomatic in the early stages and despite extensive studies for early diagnosis, there is no life-saving biomarker[2-3]. If ovarian cancer cases are detected at an early stage and treated with standard surgery and adjuvant chemotherapy, the 5-year survival rate in these patients can reach up to 90%[4]. However, although ultrasonography examination and serum cancer antigen-125 (CA-125) test are the most widely used methods in the screening of epithelial ovarian cancer (EOC), which is one of the ovarian cancer subtypes, they cannot show good effect due to low sensitivity and specificity, especially in early-stage EOC cases[5].

Extracellular proteases such as cytokines, growth factors, angiogenic factors and extracellular proteins that play a role in biological functions also play essential roles in tumor invasion, metastasis, immunity and drug resistance[6-8]. Netrin family members, including Netrin-3, Netrin-4, Netrin-5, including Netrin-1, the prototype of the netrin family, are proteins secreted by the cell to support axon growth and serve as axonal guide molecules during neural development. Netrin family proteins were first discovered in 1990 in a nematode, Caenorhabditis elegans, and are similar in structure to laminin[9]. Recent studies have shown that netrin-1, the prototype protein of this group, plays a role in many functions such as adhesion, motility, angiogenesis, apoptosis, proliferation and

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even tumorigenesis of cells in tissues such as breast, pancreas, lung and ovary, besides the nervous system[10-11]. Studies in the literature have shown that Netrin-1 is overexpressed in many cancer tissues such as kidney, liver, prostate, brain meningioma, pituitary adenoma, glioblastoma, breast cancer, and it is also released into the circulation of these patients at a high rate[12]. While it has been shown that Netrin-1 is strongly expressed in malignant ovarian tumors and cannot be detected in non-tumor tissues[13], there has been no research showing that this protein is released into the bloodstream or that it may be a biomarker in these patients.

This study aimed to determine whether netrin-1 is released into circulation by ovarian tumors and to investigate whether circulating netrin-1 can be used as a diagnostic biomarker for ovarian cancer.

## MATERIALS AND METHODS

This research is a cross-sectional type descriptive study consisting of cases diagnosed with an adnexal mass who applied to the Gynecological Oncology Outpatient Clinic at Sakarya University Training and Research Hospital between 01 November 2019 and 01 November 2020. All gynecological examinations and transvaginal ultrasonography evaluations of the patients who applied to the outpatient clinic were performed by a single doctor. Preoperatively, 8 milliliters of blood were collected by venipuncture in citrate gel and serum vacutainers, using standard procedures, from patients who agreed to be included in the study and decided to operate for the etiology of adnexal mass. Blood samples were delivered to biochemistry laboratories within 30 minutes following the established procedures. Preoperative age, body mass index (BMI) and total blood count values were recorded. After the operation, the excised tissue was examined by an experienced pathologist in terms of histopathology and grade. Patients accepted as malignant after the differentiation of benign and malignant were staged according to the International Federation of Gynecology and Obstetrics (FIGO) 2014 standards (I-IV).

Written informed consent was obtained from all patients before the study. Sakarya University Ethics Committee approved this study with issue 04 on 23/10/2019 following the World Medical Association Code of Ethics (Helsinki Declaration) published in the British Medical Journal (July 18, 1964).

### Inclusion criteria

The trial involved patients between the ages of 40 and 75 who had been diagnosed with EOC.

### Exclusion criteria

Patients with a previous diagnosis of endometrioma, borderline ovarian tumor or non-epithelial ovarian tumor, patients with another cancer, heart failure, chronic renal failure and neurological disease, and smokers were excluded from the study.

### Biochemical evaluation

The blood samples taken from the patients were centrifuged at 4000 rpm for 10 minutes, then the serums were separated and stored at -80 °C until the biochemical study day. All samples were thawed in the same month on the study day and included in the study. Netrin1- levels of the patients were measured by ELISA method using Bioassay Technology Laboratory (Zhejiang, China) kits. The kit's intra-measurement coefficient of variation (CV) was <%8, and the CV between measurements was <10%. The Netrin-1 levels of the samples studied with the manual ELISA method were read and calculated using the Biotek ELX800 (USA) ELISA reader, following the manufacturer's protocols.

### Statistics

The normal distribution of continuous variables in the study was analyzed with the Kolmogorov-Smirnov test and scatter plots. Normally distributed continuous variables were defined as mean and standard deviation, non-normally distributed variables were defined by median $25^{th}$ and $75^{th}$ quartiles, and categorical variables were made with percentages and numbers. Student t test was used to compare normally distributed variables and Mann Whitney U test was used to compare non-normally distributed variables. The distribution of netrin

**Table 1:** Comparison of some features and netrin levels of benign and malignant adnexal masses.

| Features | Benign (n=43) Median (25. pc-75.pc) | Malignant (n=40) Median (25. pc-75.pc) | *P* |
|---|---|---|---|
| Age (year) (Mean ± SD) | 56.72 ± 12.61 | 56.57 ± 13.04 | 0.959* |
| BMI (kg/m$^2$) (Mean ± SD) | 33.42 ± 4.19 | 34.18 ± 3.71 | 0.387* |
| Hemoglobin (g/dL) | 12.60 (11.50-13.60) | 12.80 (11.80-13.67) | 0.645** |
| Netrin-1 (pg/mL) | 244.00 (180.00-295.00) | 349.50 (223.00-743.00) | 0.003** |

Pc: percentile; SD: standard deviation; *Student t test; **Mann Whitney U test

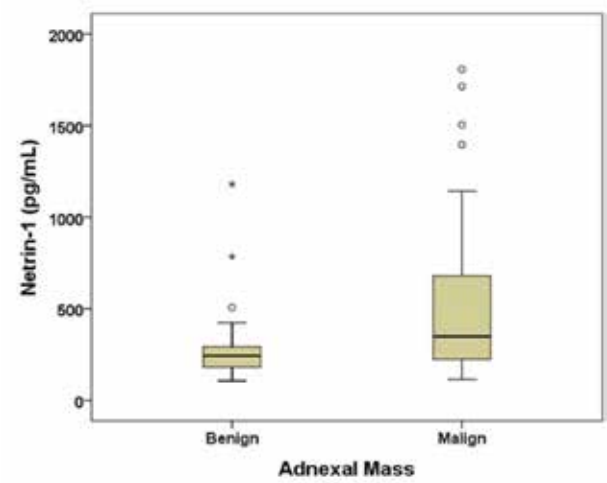


**Figure 1:** Distribution of netrin-1 levels of adnexal masses according to whether they are malignant or benign.

levels in individuals with benign and malignant adnexal masses was shown with a box plot graph. The ROC curve was used to differentiate netrin level from malignant, benign adnexal masses and to determine its sensitivity and specificity. The Youden index was used to determine the most appropriate cut-off value. Logistic regression analysis was used to determine the relationship between ovarian cancer and related features with the Netrin-1 cut-off value. SPSS 20.00 package program was used in the analyses, and $P<0.005$ was taken as the statistical significance value.

## RESULTS

A total of 83 women who were scheduled for an operation due to an adnexal mass were included in the study (Table 1). Forty-three patients operated on had benign, and 40 had malignant histopathology. The patients' ages, BMI and hemoglobin levels in both groups were similar, and there was no statistically significant difference ($P=0.959$, $P=0.387$, $P=0.645$, respectively). The median age in both groups was 56 years. The netrin level in the group with adnexal mass with malignant histopathology was higher than the group with benign histopathology, and a statistically significant difference was found between the two groups ($P=0.003$) (Table 1). In addition, adnexal masses with malignant histopathology have a wider distribution range and higher values (Figure 1).

At the end of the histopathological examination, 20% of the patients with malignant histopathology were diagnosed as Stage 1, 12.5% as Stage 2, and 67.5% as Stage 3. Of the cases with adnexal mass with malignant histopathology, 52.5% were identified as Stage 3c, 15% as Stage 1a, and 10% as Stage 3b (Table 2).

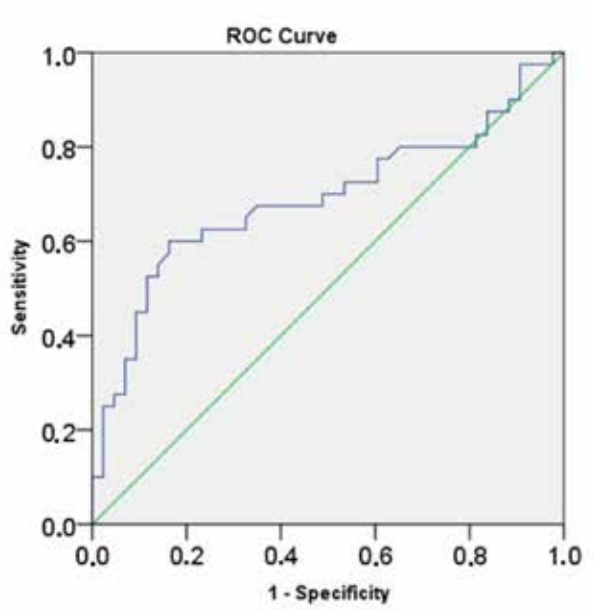


**Figure 2:** Netrin-1 level ROC curve

AUC=0.690, $P=0.003$ (95% CI: 0.571-0.809) to differentiate netrin-1 level from malignant, benign adnexal masses. According to the Youden index value in the differentiation of malignant and benign, the netrin level of 325 pg/mL and above was determined as high, and the values below it were determined as low. When the cut-off value of 325 pg/mL was accepted as the serum level in the differentiation of netrin level from malignant to benign, the sensitivity was 60%, and the specificity was 83.7% (Figure 2).

Binary logistic regression analysis was performed to analyze the cut-off value determined for all adnexal cases and the level of high (≥325 pg/mL) and low netrin values predicting malignant histopathologies. The risk of high netrin level (≥325 pg/mL) is 11.58 times higher in stage III than in stages I-II. Age, Hb level and BMI were not determinative features of high netrin level (Table 3).

**Table 2:** Distribution of stages of patients diagnosed as malignant.

| Malignant adnexal masses | Patient | |
|---|---|---|
| Stage | n | % |
| 1a | 6 | 15.0 |
| 1c | 2 | 5.0 |
| 2a | 3 | 7.5 |
| 2b | 2 | 5.0 |
| 3a | 2 | 5.0 |
| 3b | 4 | 10.0 |
| 3c | 21 | 52.5 |
| Total | 40 | 100.0 |

**Table 3:** Odds ratio of plasma netrin-1 for ovarian cancer, by age, hemoglobin, BMI and stage

| Features | B | S.E. | p | OR | 95% C.I.for EXP(B) | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| Age (year) | 0.015 | 0.031 | 0.616 | 1.015 | 0.956 | 1.078 |
| Stage | | | | | | |
| Stage I-II | reference | reference | reference | reference | | |
| Stage III | 2.450 | 0.849 | 0.004 | 11.587 | 2.195 | 61.170 |
| Hb (g/dL) | 0.332 | 0.375 | 0.376 | 1.394 | 0.669 | 2.905 |
| BMI (kg/m$^2$) | 0.024 | 0.110 | 0.830 | 1.024 | 0.825 | 1.271 |
| Constant | -7.115 | 5.707 | 0.212 | 0.001 | | |

## DISCUSSION

This study reveals that patients diagnosed with EOC have higher plasma netrin-1 values than benign ovarian tumors. In addition, it was determined that high plasma netrin-1 value was more associated with patients with advanced-stage EOC. Finally, in line with these findings, it was thought that serum netrin-1 value could be used as a biomarker to diagnose ovarian cancer.

Netrin-1, formerly known as a neurological guide factor, is receiving increasing attention for its role in tumor development. Netrin-1 expression is thought to be upregulated in some tumors and may be a potential diagnostic biomarker for human cancers.

In a study in which cancerous tissue samples and para-carcinoma samples of 86 patients diagnosed with gastric cancer were collected, it was observed that netrin-1 expression increased in gastric cancer tissues[14]. It has been shown that netrin-1 levels increase in cancerous tissues and para-carcinoma samples of 72 patients with renal clear cell carcinoma and that netrin-1 plays an essential role in forming and developing renal clear cell cancer[15]. In another study, it was concluded that 115 patients diagnosed with colorectal cancer had an increase in cancer tissues and serum netrin-1 values compared to the average population, and that netrin 1 could be used as a biomarker in patients with colorectal carcinoma[16]. In addition, it has been shown that netrin-1 plays a role in the growth, invasion and angiogenesis of ovarian cancer cells[17].

It has been shown at tissue and plasma levels that netrin-1 and netrin-1 receptors play a role in cancer development and metastasis in breast, prostate, liver cancers and meningiomas[12].

Overexpression of netrin-1 or loss of netrin-1 receptor (UNC5A-B-C, DCC) expression plays an essential role in the formation and progression of cancer[18]. Netrin-1 receptors regulate apoptosis. In the absence of netrin-1, the cell undergoes apoptosis, while in the presence of netrin-1 the cell survives. This is referred to as the 'addiction receptor' concept.

While p53 does not bind to the netrin-1 UNC5B receptor to eradicate the DNA damaged cell, it increases the transcription of UNC5B, resulting in induction of apoptosis. On the one hand, p53 increases UNC5 B transcription to induce apoptosis; on the other hand, it increases the expression and secretion of netrin-1 and decreases the progression to apoptosis through negative feedback[19-20].

Netrin-1 inhibits p53-induced apoptosis while binding to UNC5B, defined as the p53 target gene p53RDL1[21]. It has been shown that DCC induces apoptosis in the absence of netrin-1 ligand but inhibits apoptosis when combined with netrin-1[22]. Netrin-1 binds to the DCC receptor, releasing the APPL protein, which acts as an adapter between AKT2 and phosphatidylinositol 3-kinase. This leads to the activation of the AKT signaling pathway while blocking caspase-9 activation and mitochondrial-mediated apoptosis. It has also been shown in gastric cancer that it can lead to transcriptional activation of anti-apoptotic genes by inducing mitochondrial-induced apoptosis and activation of the Nuclear factor kappa-B (NF-κB) signaling pathway[1,4,23]. It has been shown that the apoptotic functions of netrin-1 receptors can be canceled by binding with netrin-1[22].

Abnormal expression of tumor suppressor genes plays an essential role in the occurrence and spread of gynecological cancers. Although the etiology of ovarian cancers is still unclear, multiple genetic alterations are known, including activating proto-oncogenes and inactivating tumor suppressor genes. TP53, a tumor suppressor gene, is the most frequently mutated gene in many cancers[24]. In particular, the TP53 mutation rate is highest in high-grade serous ovarian cancer, with 95% to 100%[25].

It has been shown in many studies that netrin-1 expression and loss of expression of netrin-1 receptors DCC and UNC5B play an essential role in the development and progression of ovarian cancer[13,20,26].

However, any study showing the plasma level in ovarian cancer has not yet been found in the literature. Our study is the first in this area, and the detection of higher plasma netrin-1 levels in patients with malignant

histopathology compared to patients with benign histopathology led us to conclude that the presence of tumor cells is associated with plasma netrin-1 level. In our study, plasma netrin-1 levels were found to strongly correlate with the tumor stage, reinforcing this result (Table 3). However, no correlation was found with plasma netrin-1 level, BMI and age. This suggests that plasma netrin-1 is an independent predictor of the presence of a tumor (Table 1). In order to prevent our results from being affected by other variables, smokers (previous studies have found high plasma netrin-1 levels in smokers) were not included in the study[27].

Fang *et al*, in their study with netrin-G1 from the Netrin family member and investigating its relationship with netrin in cisplatin-resistant ovarian tumors, showed that netrin-G1 was increased in resistant patients and decreased in non-resistance group, and inhibition of NTNG1 overcame cisplatin resistance. It was concluded that modulation of the netrin signaling pathway may contribute to improving treatment response in resistant ovarian tumors[28].

Evaluation of netrin-1 levels may be recommended in predicting the results of serous ovarian cancer treatment. Although another study showed an increase in plasma netrin-1 levels in ovarian masses associated with endometriosis, studies show that there is no difference in netrin-1 levels with the control group[29-30]. Since no clear conclusion was reached regarding plasma netrin-1 levels in endometriosis patients, our study did not include the patient group diagnosed with endometriosis.

### Limitations of the study

An immunohistochemical examination was not carried out on any of our patients. The prognostic value of netrin-1 for ovarian cancer has not been studied. Netrin-1 levels were not measured after the operation and after chemotherapy treatment. In addition, larger sample sizes would be valuable, and a similar study with a larger sample size is planned for this.

## CONCLUSION

Surgery and chemotherapy are two main treatment methods that support each other in treating ovarian cancer. However, due to chemotherapeutic drug resistance in most patients, both recurrence is seen, and the chance of surgical intervention is reduced. Introducing new pathways such as netrin-1 in ovarian tumor development may provide superiority in discovering new diagnostic and treatment strategies to improve survival and fight against cancer.

## ACKNOWLEDGMENT

**Ethics committee approval:** Our study was approved by the Local Ethics Committee of Sakarya University Faculty of Medicine (protocol ID: 11.10.2019-04)

**Informed consent:** Informed consent forms were signed by all patients who participated in the study.

**Data availability:** The study data presented may be made available from the corresponding author upon reasonable request.

**Conflicts of interest:** The authors report no conflict of interest.

**Financial disclosure:** The authors declared that this study received no financial support.

**Author contributions:** Osman Kose: conception, design, interpretation or analysis of data, preparation of manuscript, revision for important intellectual content and supervision; Elif Kose: design, interpretation or analysis of data, revision for important intellectual content and supervision; Mehmet Suhha Bostanci: conception, design, preparation of manuscript, revision for important intellectual content and supervision; Koray Gok: conception, preparation of manuscript, and supervision; Hilal Uslu Yuvaci: interpretation or analysis of data, revision for important intellectual content; Sezen Irmak: interpretation or analysis of data, preparation of manuscript and supervision

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Original Article

# Double-expressor lymphoma: Prevalence and clinicopathological features among diffuse large B cell lymphoma patients in Saudi Arabia

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## ABSTRACT

**Objectives:** To examine the clinicopathological characteristics of diffuse large B-cell lymphomas (DLBCL) and double-expressor lymphoma (DEL) and to determine the prevalence rate of DEL among patients with DLBCL.
**Design:** Retrospective study
**Setting:** University hospital in Saudi Arabia
**Subjects:** Patients diagnosed with DLBCL between January 2015 and February 2022 were identified from the hospital laboratory database. We excluded patients with primary DLBCL of the central nervous system, primary testicular DLBCL, Epstein-Barr virus-positive DLBCL or Burkitt lymphoma.
**Intervention:** Immunohistochemical stains assessment for CD3, CD20, CD10, CD5, Cyclin D1, Ki-67, BCL2, BCL6, MUM1 and c-MYC.
**Main Outcome Measures:** Clinical and immunohistochemical characteristics of DLBCL and DEL patients including the mean age, the involved site, sex distribution, proportions of CD30, BCL6, MUM1, c-MYC, BCL2, mean proliferation index (by Ki-67) and cell-of-origin subtypes. Identify the proportion of DEL within DLBCL patients and compare the differences between DEL and non-DEL groups.
**Results:** A total of 93 patients were included. The average age of DLBCL patients was 57 years. Forty-three cases (46%) were germinal center-origin DLBCL, and 50 cases (54%) were non-germinal center-origin DLBCL. Twenty patients (21.5%; average age, 59 years) had DEL phenotype. The average Ki-67 index was higher in the DEL group than in the non-DEL group. DEL was mostly associated with the germinal center-origin subtype.
**Conclusion:** We found a significant proportion of DEL cases among DLBCL cases. Therefore, immunohistochemical staining for c-MYC and BCL2 is recommended to identify this phenotype.

**KEY WORDS:** BCL2, c-MYC, diffuse large B-cell lymphomas, double-expressor lymphoma

## INTRODUCTION

Diffuse large B-cell lymphoma (DLBCL) is a neoplasm of medium-to-large B lymphoid cells with a diffuse growth pattern. It is a heterogeneous disease with many subtypes and variants recognized by their morphology and gene expression profiles (GEPs)[1]. Based on GEP studies, DLBCL is classified according to the cell-of-origin (COO) into germinal center B-cell-like (GCB) and activated B-cell-like subtypes, with a subset of cases remaining unclassifiable. However, this classification is clinically relevant, as patients with the GCB subtype have better overall survival and response to treatment[2]. Immunohistochemical staining can be used as a surrogate method to determine the COO with a good concordance rate[3,4]. The WHO classification of hematopoietic and lymphoid neoplasms describes patients with c-MYC and BCL2 rearrangements as those having high-grade B-cell lymphoma (HGBCL)[5]. HGBCLs are commonly called double-hit lymphomas (DHLs) if both rearrangements are present (c-MYC and BCL2). These patients have been found to have poor outcomes than patients with DLBCL without the rearrangement[6,7].

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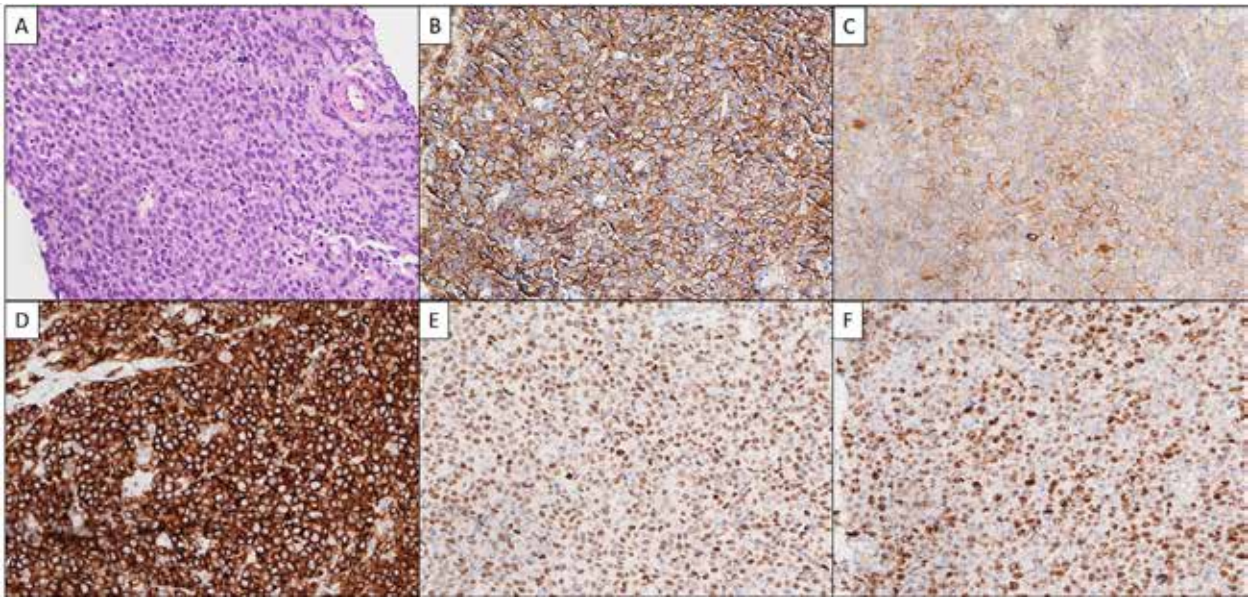


**Figure 1:** A) Hematoxylin and eosin-stained section shows sheets of large atypical cells. B) CD20 shows diffuse membranous staining in the lymphoma cells with co-expression of C) CD10. D) BCL2 shows diffuse and strong membranous staining. E) C-Myc shows increased nuclear expression in 80% of lymphoma cells. F) Ki-67 shows 80% proliferation index.

Another important prognostic subgroup of DLBCL called double-expressor lymphoma (DEL) is defined as DLBCL associated with co-expression of c-MYC (≥40% of lymphoma cells) and BCL2 (≥50% of lymphoma cells), identified using immunohistochemical staining[5]. This subgroup is associated with outcomes worse than those of non-DEL (NDEL)[8,9]. Moreover, DEL is associated with an increased risk of relapse of central nervous system (CNS) disease[10].

DLBCL is the most common type of lymphoma worldwide and in Saudi Arabia[11]. There is no study that evaluates clinical and pathologic characteristic of DEL phenotype among DLBCL patient in Saudi population. In the present study, we aimed to evaluate the frequency of DEL among DLBCL patients and clinicopathological characteristics of DEL at a tertiary center.

## MATERIALS AND METHODS

This retrospective study was conducted at King Khalid University Hospital. Ethical approval was obtained from the institutional review board before conducting the study. The hospital laboratory database was searched for patients diagnosed with DLBCL between January 2015 and February 2022. Patients with primary DLBCL of the CNS, primary testicular DLBCL, Epstein-Barr virus-positive DLBCL or Burkitt lymphoma were excluded from this study. Hematoxylin and eosin-stained sections and a panel of immunohistochemical stains, including CD3, CD20, CD5, Cyclin D1 and Ki-67 were used for diagnosis. The Hans algorithm was used to determine the COO. CD10, BCL6 and MUM1 are considered positive if ≥30% of tumor cells stain positive for each. c-MYC was considered positive if it was expressed in ≥40% of tumor cells, and BCL2 was considered positive if it was expressed in ≥50% of tumor cells. Cases co-expressing c-MYC and BCL2 were defined as DEL cases (Figure 1).

Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 25.0 (IBM-SPSS, Armonk, New York, USA). Categorical variables are expressed as numbers and percentages, and continuous variables are expressed as mean (standard deviation) values. An independent sample t-test was performed to compare the mean age and mean proliferation index between DEL and NDEL groups. Fisher's exact test was performed to compare the significant differences in the proportion of positive and negative results for the CD30, BCL6, MUM1, c-MYC, BCL2 and COO subtypes. The chi-square test was used to determine significant differences in the involved site, age groups and sex distribution between DEL and NDEL groups. A *P*-value <0.05 was considered statistically significant.

## RESULTS

A total of 93 patients with a confirmed diagnosis of DLBCL were identified during the 6-year period. Sixty-five patients were male and 28 were female (male: female ratio, 2.3:1), with an average

**Table 1:** General characteristics of patients with diffuse large B-cell lymphoma (N = 93).

| Variable | Mean (standard deviation) | n (%) |
|---|---|---|
| Age (years) | 57.29 (17.3) | |
| Age (range: minimum–maximum) (years) | 11.0–89.0 | |
| Age group (years) | | |
| <30 | | 7 (7.5) |
| 30-50 | | 18 (19.4) |
| >50 | | 68 (73.1) |
| Sex | | |
| Male | | 65 (69.9) |
| Female | | 28 (30.1) |
| Site | | |
| Nodal | | 41 (44.1) |
| Extranodal | | 52 (55.9) |
| Proliferation index | 81.51 (15.9) | |
| ≤50% | | 10 (10.8) |
| 51%-85% | | 29 (31.2) |
| >85% | | 54 (58.1) |
| CD30 | | |
| Positive | | 8 (8.6) |
| Negative | | 85 (91.4) |
| BCL6 | | |
| Positive | | 78 (83.9) |
| Negative | | 15 (16.1) |
| CD10 | | |
| Positive | | 35 (37.6) |
| Negative | | 58 (62.4) |
| MUM1 | | |
| Positive | | 68 (73.1) |
| Negative | | 25 (26.9) |
| Subtype (cell-of-origin) | | |
| GC | | 43 (46.2) |
| NGC | | 50 (53.8) |
| c-MYC | | |
| Positive | | 25 (26.9) |
| Negative | | 68 (73.1) |
| BCL2 | | |
| Positive | | 65 (69.9) |
| Negative | | 28 (30.1) |
| Double-expressor status | | |
| DEL | | 20 (21.5) |
| NDEL | | 73 (78.5) |

DEL: double-expressor lymphoma; GC: germinal center; NDEL: non-double-expressor lymphoma; NGC: non-germinal center

age of 57 years. A total of 41 cases (44%) were diagnosed based on nodal tissue. Based on the Hans algorithm, 43 cases (46%) were GCB-origin DLBCL and 50 (54%) were non-GCB-origin DLBCL. The average proliferation index obtained using Ki-67 immunostaining was 81%. Twenty-five (27%) cases were c-MYC-positive, with 20 (21.5%) cases showing co-expression of c-MYC and BCL2 (DEL). CD30 expression was identified in eight (9%) of the total patients. The general characteristics of patients with DLBCL are summarized in Table 1.

The average age of the patients with DEL was 59 years, with a male predominance. In the DEL group, 65% of patients had extranodal disease. DEL was more frequently identified in patients with the GCB subtype (65%) than in patients with the non-GCB subtype. The average proliferation index in the DEL group was 89% compared with 79.5% in the NDEL group ($P$ = 0.021). One patient with DEL was positive for CD30 expression. The results for DEL and NDEL groups are summarized in Table 2.

## DISCUSSION

According to the International Lymphoma Group, DLBCL is the most common type of non-Hodgkin lymphoma worldwide[12]. We evaluated the clinicopathological characteristics of DLBCL and the prevalence of DEL among patients with DLBCL. In the present study, the average age of patients was 57 years, which was lower than the reported age of 64 years. Furthermore, the present study revealed a marked male predominance[12]. Reportedly, 70% of DLBCL occur at nodal sites[13]. However, in the present study, only 44% of cases had nodal involvement. In addition, CD30 expression was observed in 8.6% of cases, which was less than the reported average of 14%[14].

We found that the prevalence rate of DEL (21.5%) among the newly diagnosed DLBCL cases was within the range of 20-35% reported by the previous studies[15-17]. The median age of patients with DEL was 59 years, which was not significantly different from that of patients with NDEL. In the present study, patients with DEL were younger than those included in previous studies[9]. Unlike the DEL cases in previous studies which were usually associated with non-GCB subtype, DEL cases in the present study were commonly associated with the GCB subtype[18]. There was no significant difference in sex or involved site between DEL and NDEL groups. The DEL group had a higher proliferation index (89%) than that of the NDEL group (79%).

HGBCL with c-MYC and BCL2 rearrangement (DHL) is known to have aggressive clinical behavior compared with that of DLBCL with no such rearrangement[6,7]. However, DEL without rearrangement of c-MYC and BCL2 was also found to share similar unfavorable DHL biology[9,18]. Compared with non-DHL or NDEL DLBCL, DEL is associated with poor performance status, advanced-stage disease, a high proliferative index, intermediate/high-risk to high-risk IPI scores, multiple extranodal sites of disease, and a poor complete response rate to R-CHOP chemotherapy. One study showed that an intense regimen, such as R-EPOCH, was associated with a lower relapse rate than that of the R-CHOP regimen[19].

**Table 2:** Comparison between double-expressor lymphoma and non-double-expressor lymphoma groups.

| Variable | DEL (N = 20) | NDEL (N = 73) | *P*-value |
|---|---|---|---|
| Age, mean (standard deviation), years | 58.60 (19.2) | 26.93 (16.9) | 0.705* |
| Age group (years), n (%) | | | 0.884** |
| <30 | 2 (10.0) | 5 (6.8) | |
| 30-50 | 4 (20.0) | 14 (19.2) | |
| >50 | 14 (70.0) | 54 (74.0) | |
| Sex, n (%) | | | 0.784** |
| Male | 15 (75.0) | 50 (68.5) | |
| Female | 5 (25.0) | 23 (31.5) | |
| Site, n (%) | | | 0.449** |
| Nodal | 7 (35.0) | 34 (46.6) | |
| Extranodal | 13 (65.0) | 39 (53.4) | |
| Proliferation index, mean (standard deviation), percentage | 88.75 (7.9) | 79.52 (17.0) | **0.021*** |
| CD30, n (%) | | | 0.452*** |
| Positive | 1 (5.0) | 7 (9.6) | |
| Negative | 19 (95.0) | 66 (90.4) | |
| BCL6, n (%) | | | 0.511*** |
| Positive | 18 (90.0) | 60 (82.2) | |
| Negative | 2 (10.0) | 13 (17.8) | |
| CD10, n (%) | | | **0.035***** |
| Positive | 12 (60.0) | 23 (31.5) | |
| Negative | 8 (40.0) | 50 (68.5) | |
| MUM1, n (%) | | | 0.77*** |
| Positive | 12 (60.0) | 54 (74.0) | |
| Negative | 8 (40.0) | 19 (26.0) | |
| Subtype (cell-of-origin), n (%) | | | 0.077*** |
| GC | 13 (65.0) | 43 (58.9) | |
| NGC | 7 (35.0) | 30 (41.1) | |

DEL: double-expressor lymphoma; GC: germinal center; NDEL: non-double-expressor lymphoma; NGC: non-germinal center
*Mean (standard deviation); independent samples t-test
** Chi-square test was applied
*** Fisher's exact test was applied
*P*-value was considered significant at $P < 0.05$; significant *P*-values are in bold font.
Expected outcome: DEL occurs in older age groups and is associated with a high Ki-67 index and GC subtypes.

C-MYC is a proto-oncogene that promotes cell-cycle progression and cell proliferation[20]. BCL2 inhibits cell apoptosis and promotes survival[21]. In patients with DLBCL, the deregulation of both genes results in a synergistic effect, leading to rapid lymphoma progression. This deregulation can result from translocations, mutations, copy number variations or transcriptional upregulation[22]. Fluorescence in situ hybridization (FISH) studies can detect structural abnormalities of C-MYC and BCL2 (DHL); however, other abnormalities that do not affect the structural integrity of the gene but result in their deregulation, eventually causing overexpression of these proteins can be missed, which can be detected by immunostains[23]. Therefore, a subset of patients with DHL biology is missed when relying solely on FISH studies.

## CONCLUSION

The current study shows a significant proportion of DEL phenotype among DLBCL cases. Therefore, c-MYC and BCL2 immunostaining is cost-effective for identifying cases of DEL phenotype, and we recommend its use as a standard investigation. However, our study focused primarily on the pathological and general clinical characteristics of DEL, excluding treatment outcomes. This limitation underscores the need for future studies incorporating comprehensive clinical data to elucidate the full impact of DEL on patient response to therapy and overall survival.

## ACKNOWLEDGMENT

**Limitations:** Single institution study.
**Conflict of interest:** None.

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Original Article

# Relationship between microvascular density and vascular endothelial growth factor levels and the efficacy of microwave ablation combined with anlotinib in the treatment of hepatic oligometastases in gastric cancer

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## ABSTRACT

**Objective:** This research was to investigate the relationship between the levels of microvascular density (MVD) and vascular endothelial growth factor (VEGF) and the efficacy of microwave ablation combined with Anlotinib in treating hepatic oligometastases in gastric cancer.
**Design:** 110 patients with hepatic oligometastasis of gastric cancer diagnosed in our hospital from 2019 to 2021 were rolled into a control group and an observation group under random number expression method, with 55 cases in each group.
**Setting:** All patients in the control group received total gastrectomy or subtotal gastrectomy, and some patients were combined with D2 lymph node dissection after surgery.
**Subjects:** 61 male patients and 49 female patients, aged 50-75 years old.
**Intervention:** The subjects in the observation group were additionally treated with Anlotinib hydrochloride capsules.
**Main Outcome Measure:** MVD and VEGF levels
**Results:** The total effective rate was 69% in the observation group and 34% in the control group after 3 months. After 3 months of treatment, the serum VEGF level in the observation group was 52.86±9.32 pg/mL and MVD was 67.54±7.11 strips/200 times of field of vision, while the serum VEGF level in the control group was 60.34±10.34 pg/mL and the MVD was 75.23±8.05 strips/200 times of field of vision. The postoperative survival time of patients in the observation group was 15.79±3.21 months, while that of patients in the control group was only 13.54±2.36 months ($P$ <0.05).
**Conclusion:** Microwave ablation combined with Anlotinib treatment can effectively reduce the levels of MVD and serum VEGF in patients with hepatic oligometastases of gastric cancer and improve the therapeutic effect.

**KEY WORDS:** anlotinib, microvascular density, microwave ablation, vascular endothelial growth factor

## INTRODUCTION

Gastric cancer is a malignant tumor originating from the gastric mucosa epithelium. It usually occurs in people over 50 years of age, and its early clinical manifestations are often asymptomatic, so it is often ignored in diagnosis. Advanced gastric cancer is prone to metastasis, and hepatic oligometastasis of gastric cancer[1,2] is the transitional stage between the primary focus of gastric cancer and extensive metastasis of cancer cells. Timely local treatment, chemotherapy or radiotherapy at this special stage can show very good therapeutic effects. Therefore, effective treatment of hepatic oligometastases of gastric cancer[3-5] has become the focus of attention for patients with hepatic oligometastases of gastric cancer. From January 2019 to September 2021, 55 patients diagnosed with hepatic oligometastases of gastric cancer were treated by simple surgery and microwave ablation combined with Anlotinib in our hospital. The efficacy of both treatments and the effects on microvascular density

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(MVD), serum vascular endothelial growth factor (VEGF) level, and long-term survival were investigated. The report is as follows.

## MATERIALS AND METHODS

### Data and methodologies

*General information and grouping of cases*

A total of 110 patients with hepatic oligometastases of gastric cancer diagnosed by abdominal computed tomography (CT), magnetic resonance imaging (MRI) scan, and gastroscopy in our hospital from January 2019 to September 2021 were recruited as study objects. There were 61 male patients and 49 female patients, aged 50-75 (56.51±2.41) years old. They were randomly divided into a control group and an observation group, with 55 cases in each group. The difference in clinical data between the two groups was not considerable ($P$ >0.05), and the above study subjects all signed informed consent forms (Table 1).

*Case inclusion and exclusion criteria*

Inclusion criteria: i) patients with hepatic oligometastases of gastric cancer that were diagnosed by abdominal CT scan and gastroscopy (in accordance with the Standards of the People's Republic of China Health Industry: Diagnostic Criteria for Gastric Cancer (WS 316-2010))[6]; ii) patients with no portal vein cancer thrombus or extrahepatic metastasis; iii) patients aged 50-75 years old; iv) patients with sustained bleeding in gastrointestinal tract <50 mL.

Exclusion criteria: i) patients suffering from other malignant tumors, diseases of the blood system, or complicated with serious diseases of other important organs; ii) patients who were unable or unwilling to guarantee acceptance of follow-up observers; iii) patients with hepatic encephalopathy or psychiatric disorders who can't take drugs as prescribed by doctors; iv) patients with coagulation dysfunction and bleeding tendency; v) patients with liver function above grade C and a lot of ascites by Child-Pugh modified classification; vi) patients who had Anlotinib drug allergy or tolerance.

### Treatment methods

After hospitalization, patients in both groups underwent routine hemostasis, fasting and other symptomatic treatment. Treatment methods of the control group were as follows. Conventional surgical treatment was performed with total gastrectomy or subtotal gastrectomy. Some patients were combined with D2 lymph node dissection after surgery, and the resection of hepatic oligometastases of gastric cancer ensured that the resection margin was more than 1 cm.

Treatment methods of observation group were as follows. Anlotinib capsule (Zhengda Tianqing Pharmaceutical Group Co., LTD., National medicine approval number: H20170224, specification: 12 mg) was added on the basis of microwave ablation treatment, and 12 mg was used once a day orally, which lasted for 2 consecutive weeks, with an interval of 1 week. Before microwave ablation, routine fasting was performed for 3-6 hours, puncture sites were determined by CT, and surgical sites were routinely disinfected. The cold cycle was turned on, and the needle temperature was kept below 19 °C. Under the guidance of CT, the microwave needle was directly inserted into the ablation area of liver oligometastases. The time was set at 10-17 minutes and the energy was set at 45-60 W.

### Detection of liver function recovery and prognosis after treatment

The two groups of subjects were compared after three months of treatment. Efficacy criteria[7] was as follows: (1) Complete remission: pain, jaundice, ascites symptoms and signs disappeared, normal life was not obviously impacted, which maintained for more than 1 month; (2) Partial remission: pain, jaundice, ascites symptoms and signs were relieved, normal life was slightly affected, which maintained for more than 1 month; (3) Stable disease: other symptoms and signs were improved, pain, jaundice and ascites mild symptoms were reduced, and patients often rushed to the hospital for observation and treatment; (4) Progressed disease: other symptoms and signs were aggravated, including pain, jaundice and ascites,

**Table 1:** Clinical analysis of patients in the two groups (examples)

| Grouping | N | Male | Female | Age (years) | Time from onset to operation (h) | ALT value (μL) |
|---|---|---|---|---|---|---|
| Observation group | 55 | 32 | 23 | 56.31±2.74 | 29.96±2.56 | 503.25±3.89 |
| Control group | 55 | 29 | 26 | 56.72±2.08 | 30.27±1.88 | 502.89±2.23 |
| $T$ | | | | 0.883 | 0.724 | 0.595 |
| $P$ | | | | 0.379 | 0.471 | 0.553 |

ALT: alanine aminotransferase

**Table 2:** Comparison of therapeutic effects between the two groups after 3 months of treatment

| Group | | Control group (n=55) | Observation group (n=55) | $\chi^2$ | *P* |
|---|---|---|---|---|---|
| Case number | CR | 7 | 16 | 4.452 | 0.034 |
| | PR | 12 | 22 | 4.256 | 0.039 |
| | SD | 25 | 14 | 5.789 | 0.016 |
| | PD | 11 | 3 | 5.238 | 0.022 |
| | Total effective rate (%) | 34 | 69 | 24.522 | 0.000 |

CR: complete remission; PR: partial remission; SD: stable disease; PD: progressed disease

which required the assistance of family members and medical staff. Total response rate = complete response rate + partial response rate.

Serum VEGF level and MVD detection were as follows. A total of 3 mL peripheral venous blood was extracted and placed into sterile test tubes before treatment and 1, 2 and 3 months after treatment. Serum VEGF levels were separated (3,000 r/min, 10 min) and then detected by enzyme-linked immunosorbent assay. Before treatment and 3 months after treatment, pathological puncture was performed to take cancer focus tissue, immunohistochemical staining was performed to count microvascular conditions under a 200-fold (0.74 $mm^2$) field of vision under a high-power light microscope, and 3 fields were counted in each case. Then, MVD was calculated.

### Statistical processing analysis

All the data in this experiment were input into the statistical software SPSS 18.0 for analysis. The count data were expressed by $\chi^2$ test, and all the measured data were expressed by ($\bar{X}$±s). The comparison between groups was performed by *t*-test, and the difference was considerable if $P<0.05$.

**Table 4:** Comparison of MVD between the two groups

| Group | MVD value (strip/ 200× field of view) | |
|---|---|---|
| | Before treatment | 3 months after treatment |
| Observation group (n=55) | 95.45±9.92 | 67.54±7.11 |
| Control group (n=55) | 95.01±9.98 | 75.23±8.05 |
| *t* | 0.220 | 5.145 |
| *P* | 0.826 | 0.000 |

## RESULTS

After three months of treatment, the treatment effects of the two groups were compared, and the results are shown in Table 2. The total effective rate of the observation group was 69%, which was markedly higher than 34% of the control group. The difference was considerable ($P<0.05$) (Table 2).

Comparison of MVD and serum VEGF levels between the two groups showed that MVD and serum VEGF levels in the observation group were markedly lower than those in the control group after treatment. The comparison difference was considerable ($P<0.05$) (Tables 3 and 4).

After twelve months of treatment, the long-term survival of the two groups was compared and evaluated. The long-term survival time of the observation group was 15.79±3.21 months after treatment, which was markedly longer than that of the control group (13.54±2.36 months), and the difference was considerable ($P<0.05$) (Table 5).

## DISCUSSION

Liver metastasis of gastric cancer occurs in the progressive stage of gastric cancer. The cancer cells

**Table 3:** Comparison of serum VEGF levels between the two groups

| Group | VEGF value (pg/mL) | | | |
|---|---|---|---|---|
| | Before treatment | 1 month after treatment | 2 months after treatment | 3 months after treatment |
| Observation group (n=55) | 384.66±50.33 | 122.23±21.29 | 80.47±2.65 | 52.86±9.32 |
| Control group (n=55) | 385.32±49.21 | 145.65±50.88 | 89.56±19.76 | 60.34±10.34 |
| *t* | 0.069 | 3.149 | 3.381 | 3.985 |
| *P* | 0.944 | 0.002 | 0.001 | 0.000 |

**Table 5:** Long-term survival of the two groups

| Group | Survival rate [n (%)] | | | |
|---|---|---|---|---|
| | 2 months | 10 months | 12 months | Total survival time (month) |
| Observation group (n=55) | 50 (90.91) | 342 (76.36) | 22 (40.00) | 15.79±3.21 |
| Control group (n=55) | 40 (72.73) | 30 (54.55) | 10 (18.18) | 13.54±2.36 |
| $\chi^2$/t | 6.111 | 5.789 | 6.346 | 4.188 |
| *P* | 0.013 | 0.016 | 0.012 | 0.000 |

enter the portal vein or systemic circulation to form cancer thrombi, and spread to other organs of the body to form metastases[8,9]. Patients with liver metastasis of gastric cancer will have symptoms and signs such as abdominal pain, jaundice and ascites, the quality of life of the patients will be markedly decreased, and the long-term survival rate will plummet. Liver oligometastasis of gastric cancer[10,11] is an intermediate state of liver metastasis of gastric cancer. The limited number of metastases has organ specificity, but it does not yet have the tendency of systemic dissemination, which determines the clinical significance of local treatment. Early detection of liver oligometastasis from gastric cancer and prompt, active, rapid, and reasonable treatment can improve the quality of life of patients, reduce MVD and serum VEGF level, and save their lives. Some researchers believe that surgical resection of the liver lobe is still the optimal treatment option for liver oligometastasis of gastric cancer[12,13]. However, due to the difficulties in early diagnosis of gastric cancer, the concealment of metastasis, and the fact that it is often accompanied by metastasis of various organs outside the liver and lymph nodes, conventional surgical resection of the liver lobe does not achieve the ideal curative effect in most cases.

In this study, the subjects in the observation group were treated with microwave ablation combined with Anlotinib. Patients diagnosed with liver oligometastasis of gastric cancer by CT and MRI scans received direct ablation treatment by puncturing the liver metastases with a microwave ablation needle guided by CT. A recent randomized controlled trial showed that local ablation markedly improved the median progress free survival of patients with oligometastatic lung cancer compared with conventional surgery. The biggest feature of this minimally invasive treatment is that it has little trauma to patients, which improves the quality of life of patients, reduces the pain of treatment, and has little systemic impact. As a multi-target tyrosine kinase inhibitor, Anlotinib can inhibit tumor angiogenesis and tumor growth. The drug can be taken orally, which is convenient to use and greatly improves the quality of life of patients. Additionally, it has low pharmacological toxicity, favorable clinical effect and few adverse reactions. Clinically, it is often adopted to treat malignant tumors such as non-small cell lung cancer, gastric cancer, soft tissue sarcoma and cancer cell metastasis. MVD is a marker of angiogenesis. In the development of tumors, tumor cells will release substances such as VEGF and induce the generation of structurally and functionally abnormal microvascular block, indicating that it is an important indicator of the intensity and activity of tumor angiogenesis[14]. VEGF, also known as vascular permeability factor, is a highly specific pro-vascular endothelial cell growth factor. The MVD and serum VEGF water in patients with malignant tumor increase to various degrees, which has certain reference value for the occurrence and progression of tumor and the judgment of therapeutic effect[15]. For malignant tumors of the digestive system such as gastric cancer, MVD and serum VEGF levels in patients are related to the diameter of the primary tumor of the cancer and reflect the degree of growth and metastasis of the lesion. Hence, MVD and serum VEGF levels are closely related to the curative effect of liver oligometastasis from gastric cancer, which can reflect its curative effect. Patients in the control group were treated with conventional surgery, subtotal gastrectomy and D2 lymph node dissection, and removal of liver oligometastases. Two treatment methods were compared. After treatment for three months, most patients in the observation group had obvious alleviation of abdominal pain, jaundice and ascites symptoms and signs, and the total effective rate was 69%. In the control group, patients experienced more disease progression and aggravation of symptoms and signs after three months of treatment, and the total effective rate was 34%. The results demonstrated that microwave ablation combined with Anlotinib treatment could well improve the symptoms and control the disease. After three months of treatment, the serum levels of VEGF and MVD of patients in the observation group were markedly lower than those in the control group. This indicates that the microwave ablation combined with Anlotinib treatment can effectively control the proliferation and invasion of cancer cells and delay the progression of cancer. The patients were followed up for 2, 10 and 12

months after treatment and the data showed that the survival time of patients in the observation group was 15.79±3.21 months after operation, while that of patients in the control group was only 13.54±2.36 months. This indicates that microwave ablation combined with Anlotinib treatment can improve the survival rate and save the life of patients with gastric cancer and hepatic oligometastasis. The outcomes of patients in the observation group after the three treatments were better than those in the control group. The difference between the two groups was considerable.

Despite the potential of microwave ablation combined with Anlotinib in patients with gastric cancer and liver oligometastasis treatment, the study has significant limitations. Firstly, the study design is single-center, which may restrict the generalizability of the results. Secondly, the sample size is insufficient and separate analyses of microwave ablation and Anlotinib were not performed, nor was the impact of Anlotinib dosage on efficacy clearly elucidated. Future research should consider a multicenter design, stringent randomized double-blind controlled trials, increased sample sizes, and comprehensive subgroup analyses to fully evaluate the efficacy of microwave ablation combined with Anlotinib in patients with gastric cancer and liver oligometastasis. Additionally, extended patient follow-up, monitoring of drug side effects, and dose adjustments are needed to improve quality of life and increase survival.

## CONCLUSION

In conclusion, microwave ablation combined with Anlotinib therapy can effectively reduce the serum VEGF level of patients with gastric cancer and liver oligometastasis, improve the therapeutic effect, improve the quality of life of patients, improve the prognosis of patients, and reduce the mortality rate of patients, and thus can be widely applied in clinical practice.

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Case Report

# Rare cause of chest pain: Megaesophagus forming achalasia

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## ABSTRACT

Achalasia is esophageal motor dysfunction caused by impaired relaxation of the lower esophageal sphincter. A 77-year-old female patient was brought to the emergency department with complaints of nausea, vomiting and chest pain. Thorax computed tomography (CT) imaging was performed because there was no abnormality in troponin and electrocardiogram (ECG) follow-ups; approximately 7 cm diameter megaesophagus was detected. Endoscopy was performed for advanced diagnosis and treatment. Diagnosis of achalasia was confirmed by endoscopy. The distal end of the esophagus was widened by dilatation. The patient was discharged after 24 hours of service follow-up. Although it is a rare condition in elderly patients presenting to the emergency department, it should be noted that achalasia will constitute a non-cardiac chest pain clinic.

**KEY WORDS**: achalasia, candida, chest pain, megaesophagus

## INTRODUCTION

Achalasia is a rare esophageal motor dysfunction that occurs when relaxation of the lower esophageal sphincter is impaired. Peristaltic movements of esophageal smooth muscles are inconsistent or absent. The pressure of the lower esophageal sphincter is high in less than 50% of the patients. This causes esophageal dilatation secondary to obstruction[1,2]. Its annual prevalence is approximately 10/100000 and its incidence is 1/100000. There is no specific gender, age or race that it predominantly affects. It occurs with the same frequency in men and women. The most common age range is adults between the ages of 30 and 60. The second most common group is people over the age of 60[3,4].

In our study, we aimed to present a case that was transferred to an external center with the complaints of chest pain, epigastric pain, nausea and vomiting, and referred with the prediagnosis of acute coronary syndrome, but diagnosed with achalasia.

## CASE REPORT

A 77-year-old female patient was admitted to the district state hospital with the complaints of epigastric pain, nausea, vomiting and chest pain. She was transferred to our hospital due to the increase in her complaints. Vital signs are blood pressure arterial 100/70 mmHg, pulse 68/minute, fingertip pulse oxygen saturation 96% and fever 36.2 °C. The patient was conscious, oriented, cooperative and her Glasgow coma score was 15. She had no history of any disease other than hypertension, and she did not have a history of any surgical operation. There were no pathological findings in respiratory system examination. Her electrocardiogram was normal sinus rhythm. In the abdominal examination, significant tenderness in the epigastric region and the presence of defense, especially in the upper quadrants, were significant examination findings, and there was no rebound.

In the detailed anamnesis of the patient, it was learned that she had stomach pain and dysphagia for

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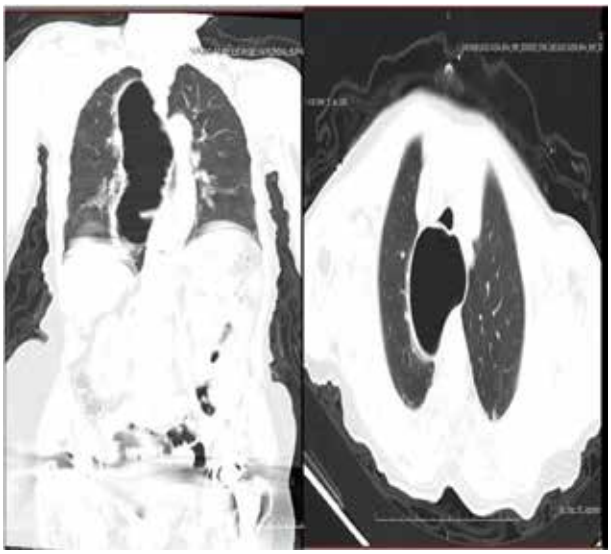

**Fig 1:** Images of the coronal and axial thorax CT of the megaesophagus; approximately 7 cm in diameter.

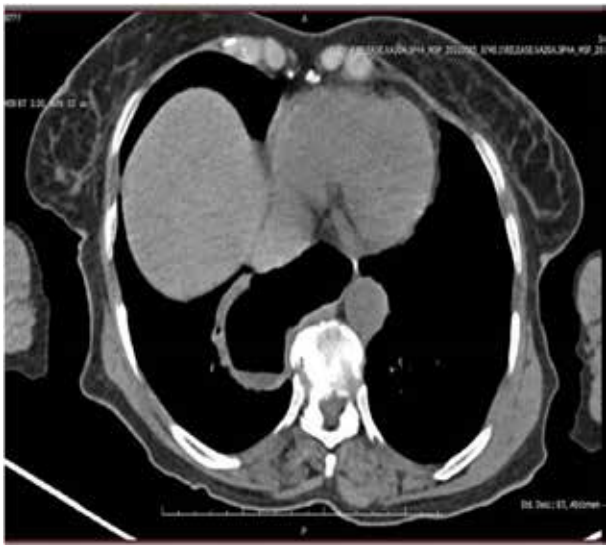

**Fig 2:** Narrowing and air-fluid leveling in the distal esophagus

years and used a proton pump inhibitor. However, she had no previous history of recurrent aspiration. When we question the character of chest pain complaint; the pain had been going on for a long time, but its severity had increased for 2 days, especially with eating solid food, the desire to eat has decreased due to this pain. As a result of this, she lost 5 kilograms in two months. In addition, she slept with 2-3 pillows at night despite the absence of a diagnosis of heart failure; otherwise, it was learned that she woke up with shortness of breath and cough.

Significant laboratory values of the patient; glucose is 195 mg/dl, blood leukocyte is 13400/ul, 1st troponin value is 19ng/L, 2nd troponin value taken after 3 hours is 12ng/L, D-dimer is 331 ng/ml.

After the current examination and detailed anamnesis, the thought of a pathology that may cause non-cardiac chest pain came to the fore in the patient, and computed tomography (CT) of the thorax and abdomen was planned. In thorax CT, we encountered an esophageal image with a width that we have never seen before. At its widest point, this megaesophagus had reached a diameter of approximately 6.75 cm (Figure 1). The patient's previous results were reviewed from the hospital database. The last chest X-ray was viewed 5 years ago and no pathological image could be detected. The distal end of the esophagus was narrowed, and air-fluid level was observed in this region (Figure 2). No pathology was found to explain the current clinic in abdominal CT images.

Consultation was requested from general surgery and radiology specialists. Emergency endoscopy was planned to establish a definitive diagnosis. Endoscopy procedure was started under anesthesia with lidocaine local spray, 5 mg midazolam, 5 mg flumazenil. Vocal cords were seen and the esophagus was entered. Esophageal motility was negligible. The cervical and thoracic esophagus was extremely wide and filled with liquid intestinal secretion. The lower esophageal sphincter was so narrow as not to allow the pass of the endoscopy probe (Figure 3). The stomach was entered by pneumatic dilation. The gastric mucosa was hyperemic. The pylorus and duodenum had a normal endoscopic appearance. Separate biopsy material was taken from the gastric mucosa and lower, middle and proximal parts of the esophagus. As a result of the biopsy, candida hyphae and spores were observed with pas-ab dye with a pH of 2.5 and diagnosed as achalasia.

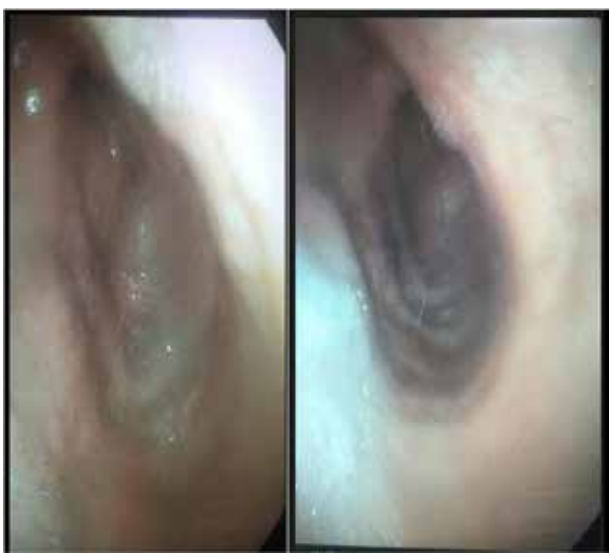

**Fig 3:** Endoscopic view of narrowing at the distal end of the esophagus

The endoscopic procedure performed had diagnostic and therapeutic features, and the patient was diagnosed with achalasia and acute gastritis, so pneumatic dilation was performed for treatment. The patient was hospitalized in the general surgery service for 24 hours for observation after endoscopy, and was discharged the next day with recovery.

In the control thorax CT performed 2 months later, the megaesophageal image persisted, but the esophageal diameter was found to have regressed. The patient said that she did not have epigastric pain, nausea, vomiting, chest pain complaints in the control examination, and she could eat comfortably.

## DISCUSSION

Achalasia patients mostly apply to the hospital with dysphagia (more than 90% of applicants), regurgitation, chest pain and respiratory failure. As the disease progresses, aspiration, nocturnal cough, heartburn and weight loss may occur[5,6]. Although our case was sent from an external center with preliminary diagnoses of acute coronary syndrome and peptic ulcus, detailed anamnesis and examination of the patient, decrease in troponin value from laboratory values after control, forced us to think of other diagnoses. Our literature reviews ruled out the possibility of acute coronary syndrome in case of decreased troponin values[7]. For the diagnosis, thoracic and abdominal imaging was performed due to the need for imaging.

An approximately 6.75 cm diameter megaesophageal image which we encountered was detected in the thorax CT mediastinal window. In the few literature reviews on the subject, it was emphasized that the presence of megaesophagus is significant for achalasia in its differential diagnosis[8]. As a result of our consultation with general surgery and radiology specialists, the diagnosis of achalasia and esophageal perforation came to the fore. The air-fluid level image at the lower end of the esophagus brought to mind the preliminary diagnosis of esophageal perforation. However, the absence of air in the mediastinum distracted us from this diagnosis. Significant decrease in esophageal peristaltic movements during endoscopy, food and secretion residues accumulating in the esophagus, and excessively narrowed lower esophageal sphincter are common in the diagnosis of achalasia[6]. Gockel *et al* explains the answer to the question of how this patient was not diagnosed with achalasia until the age of 77, as follows; in addition to the nonspecific and mild symptoms at the onset of the disease, the diagnosis of patients is usually delayed in cases where endoscopic/radiological imaging is not performed[9]. Cheung *et al*, in their study, attributed another reason for being diagnosed with late achalasia to the fact of the patients' being in a nursing home or being homeless[10]. Although the relatives of the patient did not accept, in our case, it was thought that there might be a problem related to the care of the elderly patient and dealing with the patient.

Barium esophageal radiography imaging was not attempted when the patient declared that he could not tolerate drinking barium. Esophageal manometry method[11], which is the gold standard test used to diagnose achalasia, cannot be performed in our hospital. Endoscopic procedure was performed in our patient in the operating room environment, and it was understood that the megaesophagus, which we saw on thorax CT, was caused by achalasia. Since the mass to cause obstruction was not seen on thorax-abdominal CT imaging and during endoscopy, malignancy was not considered in the patient.

Megaesophagus is the most common long-term complication seen in 253 achalasia patients followed by Eckardt *et al* for 33 years. They comment that in the presence of megaesophagus, we can guess that it has been more than 10 years since the onset of the disease[12]. The megaesophagus condition seen in our case also made us suggest that the patient had been living with this clinic for many years, but had come to this time without being diagnosed.

Co-occurrence of Candida infections and achalasia can be observed[13]. In our case also, there was candida involvement as a result of biopsy, and we think that candida involvement developed secondary to increased esophageal muscle mass, food accumulation and disruption of peristatic movements.

Non-surgical methods in the treatment of achalasia are pharmacotherapy (calcium channel blockers, nitrates, phosphodiesterase-5 inhibitors), pneumatic dilation and endoscopic botulinum toxin injection. Surgical methods are peroral endoscopic myotomy (POEM) and laparoscopic Heller myotomy (LHM) operations. Pharmacotherapy is applied to patients who do not accept pneumatic dilation or surgery, but its effectiveness is controversial[14]. Pneumatic dilation with a 3 cm balloon was applied to our case, the lower esophageal sphincter was expanded, the existing obstruction was opened. POEM and LHM were not considered as they could not be performed in our hospital. In addition, the relatives of the patients were against major surgery due to the age of the patient.

## CONCLUSION

In elderly patients applying to the emergency department with chest and stomach pain, radiological imaging should be performed to detect non-cardiac pathologies. The presence of megaesophageal image in the mediastinal window should suggest the diagnosis of achalasia. Since definitive curative treatment of achalasia is not possible, the patient should be told

that the treatment aims to increase the quality of life by reducing symptoms such as dysphagia, chest pain, shortness of breath and weight loss.

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**Authors contributions:** Mustafa Oguz Cumaoglu examined the patient in the emergency room and diagnosed him. He collected the patient's data, turned it into a case report and wrote it. Turgut Dolanbay assisted in the writing of the case report. He made an English translation of the study. Haci Bolat performed endoscopic imaging of the patient. The service followed up and discharged the patient.

**Ethical considerations:** Permission to use the patient's data in academic studies was obtained from her daughter.

**Conflict of interest and financial disclosure:** The authors declare no competing financial interests and no conflicts of interest concerning the authorship and/or publication of this article.

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Case Report

# Saddle pulmonary embolism in a patient with atypical clinical presentation: a case report

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## ABSTRACT

Saddle pulmonary embolism (PE), which is a rare form of acute PE, can cause sudden hemodynamic deterioration and death. In this case, we present a female patient who presented to the emergency department with complaints of back pain and was found to have sinus bradycardia on electrocardiogram, ultimately diagnosed with saddle PE. A chest computed tomography was ordered to evaluate for a possible lung infection. Although no evidence of pneumonia was found, the imaging showed an enlarged diameter of the pulmonary artery measuring 3.22 cm. Saddle PE is a condition that should be considered in the diagnosis in the Emergency Department as it can lead to serious mortality and morbidity, and may not always present with typical symptoms. In cases with atypical presentation where PE is not initially suspected, the high diameter of the main pulmonary artery detected on chest CT scans should be associated with diseases with right ventricular overloading such as PE.

**KEY WORDS**: bradycardia, pulmonary artery, saddle pulmonary embolism

## INTRODUCTION

Acute pulmonary embolism (PE), which is one of the types of venous thromboembolism (VTE), is a serious condition that can result in morbidity and mortality. In untreated cases of acute PE, mortality rates can be around 30%, while in treated cases, the rate is around 8%[1]. The classical triad of dyspnea, hemoptysis and pleuritic chest pain, which is commonly associated with PE, represents only 10% of cases. On the other hand, PE can mimic a wide range of clinical presentations, from a simple cough or muscle pain to a severe acute myocardial infarction. These atypical clinical presentations can make it challenging for physicians to diagnose PE[2].

Saddle PE, which is a rare form of acute PE, can cause sudden hemodynamic deterioration and death. In saddle PE, a thrombus is present at the bifurcation of the main pulmonary artery[3]. When evaluating cases of acute PE, approximately 2% to 5% of cases are diagnosed as saddle PE[4].

In this case, we present a female patient who presented to the emergency department (ED) with complaints of back pain and was found to have sinus bradycardia on electrocardiogram (ECG), ultimately diagnosed with saddle PE.

## CASE REPORT

A 77-year-old female patient was brought to our ED by 112 ambulance with complaints of back pain. According to the history obtained from the patient and her family, the patient had been experiencing back pain for one day and had no other complaints. It was reported that the patient's pain had intensified three hours before coming to the ED and did not respond to analgesics. The patient had a known diagnosis of hypertension and was taking beloc 50 mg/day besides any other medication. One week prior to admission, she was hospitalized in the internal medicine department due to a urinary tract infection (UTI) and received treatment for six days.

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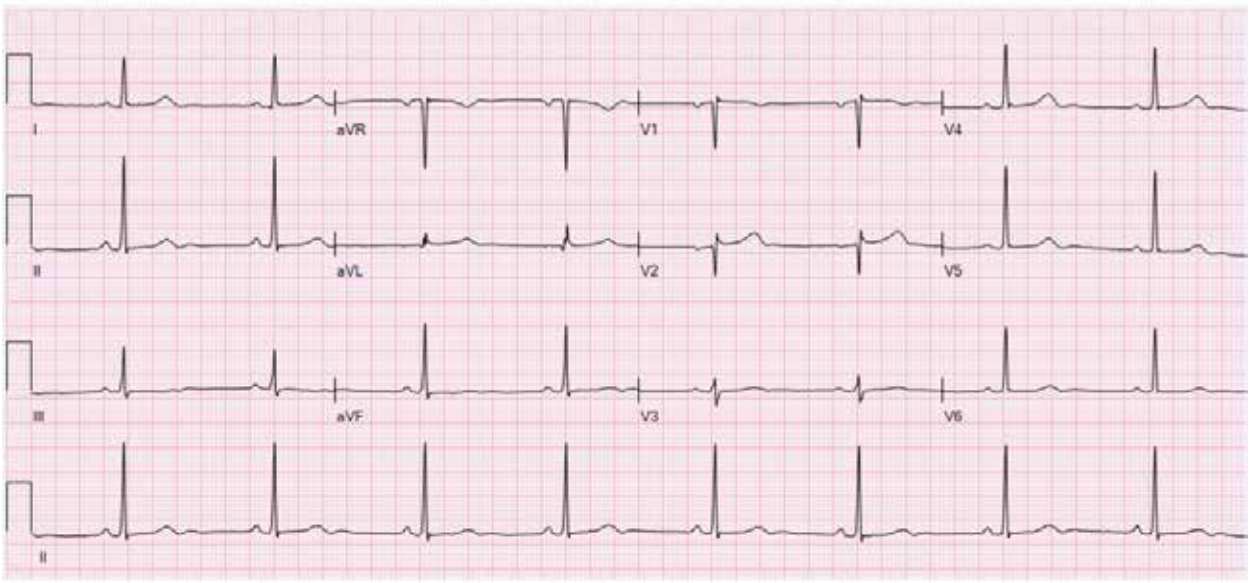


**Figure 1**: The patient's ECG shows sinus bradycardia with 50 beats/per minute.

During the physical examination, the patient was found to be in poor general condition with vital signs of arterial blood pressure: 100/60 mmHg, oxygen saturation (SpO2): 84%, temperature: 36.3°C, and pulse rate: 50 beats/minute. Systemic examinations, including neurological, cardiovascular, respiratory and abdominal examinations, revealed no pathology other than general malaise. The patient's ECG showed sinus bradycardia (50 bpm) (Figure 1).

The patient was started on O2 at 4-6 liters/minute to stabilize her vital signs. In the patient's blood tests at the time of admission, pathologically, troponin: 75 ng/L, c-reactive protein: 37.2 mg/dl was detected. In addition, 15 leukocytes, 33 erythrocytes and 2 bacteria were detected in the urinalysis. Other laboratory values of the patient are also summarized in Table 1.

Initially, the patient's symptoms were attributed to a UTI, but further investigations were necessary as her low SpO2 levels persisted despite receiving oxygen therapy. A chest computed tomography (CT) was ordered to evaluate for a possible lung infection. Although no evidence of pneumonia was found, the imaging showed an enlarged diameter of the pulmonary artery measuring 3.22 cm (Figure 2). Following suspicions of PE, a D-Dimer test was ordered and returned with high levels (8514). An echocardiography (ECHO) was also performed and revealed right ventricular dilation, prompting further investigations, including a CT pulmonary angiography (CTPA), which confirmed the diagnosis of PE with saddle-shaped filling defects in bilateral main pulmonary arteries (Figure 3).

The patient was promptly treated with unfractionated heparin in the ED and was admitted to the chest diseases intensive care unit for advanced treatment and follow-up. The patient received 5 days of unfractionated heparin infusion, followed by oral administration of 20 mg of rivaroxaban for 6 days. The patient was closely monitored in the intensive care unit for 11 days, after which the patient was discharged with a prescription for 20 mg of rivaroxaban once a day and an oxygen concentrator.

**Table 1:** Some laboratory results of the patient's hemogram, biochemistry and blood gas analysis.

| Laboratory | Lab values | References range |
|---|---|---|
| **Hemogram** | | |
| Hemoglobin (Hb) | 12.5 | 11 – 15 g/dL |
| Leukocyte (WBC) | 11.07 | 4 – 10 10^3 /ul |
| **Biochemistry** | | |
| Glucose | 126 | 74 – 10 mg/dl |
| Urea | 31 | 17 – 49 mg/dl |
| Creatinine | 1.01 | 0.5 – 0.9 mg/dl |
| Aspartate Aminotransferase (AST) | 23 | 5 – 32 U/L |
| Alanine Aminotransferase (ALT) | 18 | 5 – 33 U/L |
| Albumin | 33 | 35 – 52 U/L |
| Troponin T | 75 | 0 – 14 ng/L |
| Sodium (Na) | 134 | 135 – 145 mmol/L |
| Potassium (K) | 3.4 | 3.5 – 5.5 mmol/L |
| Chloride (Cl) | 98 | 95 – 110 mmol/L |
| C- Reactive Protein (CRP) | 37.2 | 0 – 5 mg/L |
| **Blood Gas Analysis** | | |
| pH | 7.400 | 7.35 – 7.45 |
| Bicarbonate | 17.8 | 22 – 26 mmol/L |

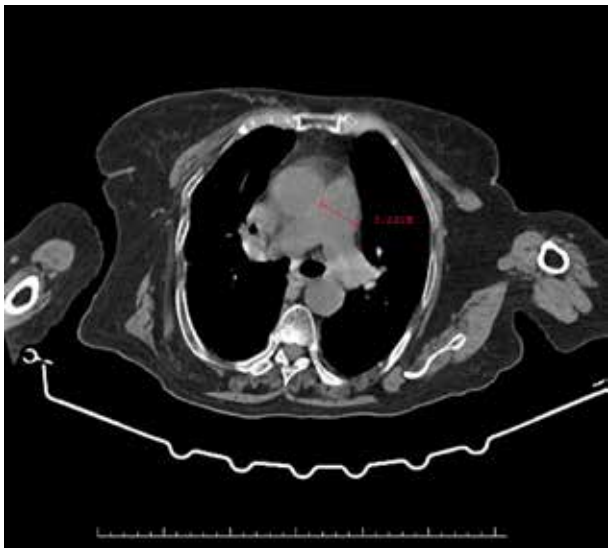

**Figure 2**: The main pulmonary artery diameter appears widened on the non-contrast chest CT result.

The symptoms of the patient, who was followed up monthly after discharge from the hospital, decreased. In this process, our patient continued on 20 mg rivaroxaban and oxygen treatment per day for 3 months. Deep vein thrombosis (DVT) was detected in the popliteal vein and deep crural veins in the doppler ultrasonography performed during the follow-up of the patient. 20 mg rivaroxaban treatment of the patient with DVT was extended to 9 months. In the ECHO performed during the follow-up of the patient; ejection fraction was 60%, first degree mitral regurgitation, mild enlargement of the left atrium, first degree tricuspid regurgitation, and pulmonary artery pressure was measured as 35-40 mmHg.

## DISCUSSION

PE is the third most common cause of cardiovascular death worldwide after stroke and heart attack[5]. Cases of PE can present to hospitals with very different symptoms depending on the severity of the embolism. It can mimic many cardiac and chest diseases and is therefore one of the frequently overlooked diseases in ED. Especially, the mortality of massive PE is very high within the first hour, and the diagnosis and treatment of this life-threatening condition should be started[6]. Hypodense filling defects are seen as the finding of thoracic CT angiography in imaging. These filling defects can be segmental, subsegmental and saddle-shaped depending on the location. This condition, which is saddle-shaped, can completely block the pulmonary artery and can create a serious clinical picture[7]. However, in our case, saddle PE presented to us with atypical symptoms such as low back pain, except for serious symptoms such as significant dyspnea or syncope.

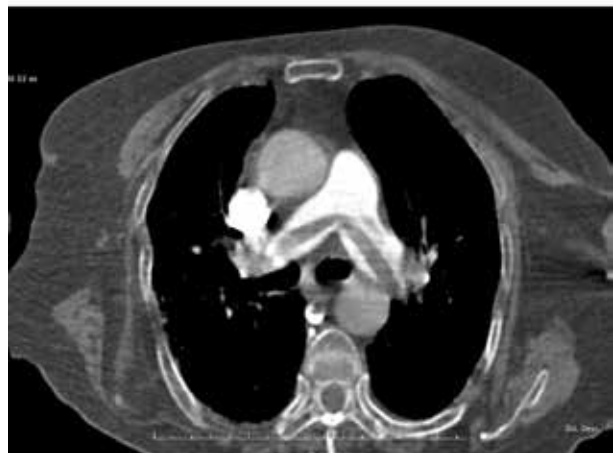

**Figure 3**: Axial section contrast-enhanced CTPA shows saddle-shaped filling defects in the bilateral main pulmonary arteries.

PE risk factors are very diverse. Hospitalization and immobilization are known risk factors for the development of DVT, which can subsequently lead to PE[8]. In addition, advanced age is also a significant risk factor for the development of PE, as the incidence of VTE increases with age[9]. Our patient had been hospitalized for 6 days in the internal medicine ward due to UTI, and the patient presented to our ED with his current condition 1 day after discharge. The most striking risk factors in our patient were recent hospitalization, immobilization and advanced age.

Sinus tachycardia is a most common finding in PE, while more severe cases can present with various ECG changes, including T-wave inversion in V1-V4 derivations, QR pattern in V1, S1Q3T3 pattern, and incomplete or complete right bundle branch block. These changes are indicative of right ventricular strain, which can occur due to increased pressure in the pulmonary arteries caused by the presence of the embolism. In our case, sinus bradycardia was detected in the performed ECG, which could be misleading in the diagnosis, and this condition was attributed to two reasons. The first is that the patient was under the treatment of beloc 50 mg/day, which is an antihypertensive drug, and the second is that the signs of right ventricular dilatation and loading may cause vagal stimulation. Cheema *et al* also attributed the cause of deep bradycardia detected in a PE case at the age of 48 to vagal stimulation developing secondary to right ventricular loading signs[10].

In studies, the wide diameter of the main pulmonary artery indicates signs of right ventricular loading[11]. In a study conducted by Ozturk *et al*, a pulmonary artery diameter higher than 2.86 cm for pulmonary arterial hypertension was reported to be 69% sensitive and 100% specific[12]. To put it in simple terms, according to this information, a high main pulmonary artery diameter detected by CTPA scan should be associated

with diseases such as PE. On the other hand, while a chest CT can provide some information regarding the lungs and pulmonary arteries, it may not be sufficient to definitively diagnose PE. However, in this case, the presence of an enlarged pulmonary artery on the chest CT raised suspicion for PE, prompting further evaluation with a CTPA. The development of acute right heart failure after pulmonary thromboembolism leads to right ventricular dilation, increasing the oxygen demand of the right ventricle. This condition reduces blood flow in the right coronary artery and results in microinfarctions in the right ventricular muscles, causing an increase in troponin release. Elevated troponin levels indicate right ventricular dysfunction and are associated with high mortality rates[13]. The patient's ECHO showed right ventricular dilation and a high troponin level, which is a finding consistent with the literature. If a patient has been diagnosed with PE or is clinically considered to be at high risk, diagnostic procedures should continue while treatment with unfractionated heparin, low-molecular-weight heparin, fondaparinux, or new generation anticoagulants is recommended. Thrombolytic therapy is also recommended in appropriate cases. Additionally, percutaneous catheter-related treatment and surgical embolectomy are recommended in the treatment of PE[14]. In our hemodynamically stable patient, medical thrombolytic therapy, percutaneous catheter-related treatment and surgical embolectomy were not considered. During their stay in the ICU, the patient was treated with new generation oral anticoagulants after intravenous anticoagulant therapy to improve blood flow and prevent further blood clots.

## CONCLUSION

Saddle PE is a condition that should be considered in the diagnosis in ED as it can lead to serious mortality and morbidity, and may not always present with typical symptoms. In cases with atypical presentation where PE is not initially suspected, the high diameter of the main pulmonary artery detected on chest CT scans should be associated with diseases with right ventricular overloading such as PE. In addition, it should be kept in mind that sinus bradycardia may rarely occur in PE.

## ACKNOWLEDGMENT

Thank you to our patient and their relatives for allowing us to use their data. In this case report, written informed consent was obtained from the patient by the author.

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Case Report

# Hyperpigmentation after hypofractionated boost irradiation following hypofractionated whole breast irradiation

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## ABSTRACT

**Background:** No cases have been reported in which the contralateral breast was treated with conventional whole-breast irradiation (WBI) while the ipsilateral breast was treated with hypofractionated WBI and a hypofractionated boost.
**Case report:** A 58-year-old woman with breast cancer underwent WBI and a boost to the tumor bed. She had a history of early-stage breast cancer on the contralateral side, for which she received conventional WBI with a total dose of 50 Gy in 25 fractions. She received a total of 39 Gy in 13 fractions and a boost with a dose of 9 Gy in 3 fractions. Mild dermatitis occurred during treatment, and mild hyperpigmentation developed 3 months after boost irradiation, which worsened over time and was considered a grade 2 late adverse event. Six years later, no recurrence or additional adverse events occurred.
**Conclusion:** Hypofractionated boost irradiation after hypofractionated WBI carries a risk of hyperpigmentation and should be treated with caution, even if conventional WBI did not cause late skin damage.

**KEY WORDS:** hypofractionated, pigmentation, radiation dermatitis, radiotherapy

## INTRODUCTION

Breast cancer patients after whole-breast irradiation (WBI) may experience complications such as skin dryness, hypersensitivity and hyperpigmentation in the irradiated skin areas, which can induce a depressive psychological state and affect their quality of life[1]. Hypofractionated WBI has become a standard fractionation method, along with conventional WBI[2]. However, there is no comparative data on late skin reactions in the same patient. Here, we report a case of bilateral early-stage breast cancer treated postoperatively with conventional WBI on one side and hypofractionated WBI on the other side, and evaluate the differences in late skin reactions.

## CASE REPORT

A 58-year-old woman with breast cancer on the right side was referred to our hospital for WBI after lumpectomy and axillary lymph node dissection. Histological evaluation revealed luminal invasive ductal carcinoma, grade 2, spanning 1.8 cm in greatest dimension (pT1cN1M0, AJCC Cancer Staging Manual. 8th ed.). Severe lymphovascular invasion, intraductal spread and fat tissue invasion were identified. Immunohistochemical staining showed tumor cells that were strongly and diffusely positive for estrogen receptor and progesterone receptor. Human epidermal growth factor receptor 2 (HER2) overexpression was equivocal on immunohistochemical staining. Subsequent fluorescence in situ hybridization for HER2 did not reveal amplification. The resection margin was negative for cancer cells, but cancer cells were present within 2 mm of the resection margin. WBI and a boost to the tumour bed was planned. This patient had early-stage breast cancer on the left side 12 years earlier and had received conventional WBI after breast-conserving surgery, so image-guided intensity modulated radiation therapy was performed to

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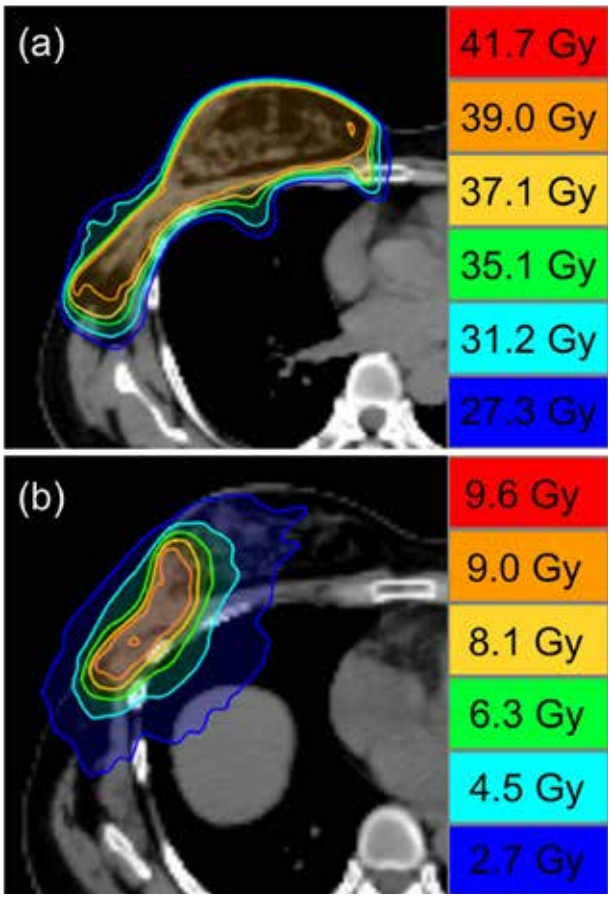


**Fig. 1:** Radiation therapy isodose lines with corresponding actual radiation dose.
(a) Whole breast irradiation plan.
(b) Boost plan.

reduce excessive radiation exposure to the previously irradiated field.

All procedures performed in this study were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Institutional review board approval was waived because every treatment was approved by the national health insurance. Informed consent was obtained from the patient for the use of clinical data. The patient had already undergone conventional WBI at a dose of 50 Gy in 25 fractions for early-stage breast cancer on the left side 12 years earlier. The post-treatment course was uneventful, with no late skin reaction or recurrence. In this case, since the national health insurance allowed hypofractionated WBI, a total dose of 39 Gy in 13 fractions was delivered using helical tomotherapy (Hi·Art® treatment system, Madison, Wisconsin, USA) (Fig. 1a). Since the cancer cells are very close to the rim of normal tissue, boost irradiation was given to the surgical bed at a dose of 9 Gy in 3 fractions without bolus (Fig. 1b). One month after radiotherapy, aromatase inhibitor therapy was initiated and continued for 5 years. The Fitzpatrick skin phototype was skin type III. During radiotherapy, mild erythema and desquamation was observed, but the patient recovered within a month without treatment. However, three months after radiotherapy, mild hyperpigmentation was observed in the tumor bed where boost irradiation was performed, which gradually worsened, and three years later, numerous patchy hyperpigmentations were evident (Fig. 2a). Furthermore, pigmentation progressed gradually over 6 years of follow-up (Fig. 2b). Based on clinical findings, these cutaneous reactions were considered Grade 2 adverse events (NCI Common Terminology Criteria for Adverse Events, v4.03). Six years after WBI and a boost, there were no recurrences or additional adverse events.

## DISCUSSION

There have been no reports of patients who received both conventional and hypofractionated WBI after breast-conserving surgery and developed different late skin reactions. Since conventional and hypofractionated WBI are comparable in terms of treatment efficacy and frequency of late adverse events, the pigmentation of the tumor bed may be due to the boost dose administered after hypofractionated WBI[1-3].

Ionizing radiation can induce hyperpigmentation through several physiological mechanisms. First, ionizing radiation stimulates melanogenesis, which produces melanin that protects against deoxyribonucleic acid (DNA) damage. Overstimulation by ionizing radiation can cause hyperpigmentation[4,5]. Second, ionizing radiation induces inflammation, activating melanocytes and increasing melanin synthesis, leading to hyperpigmentation[6]. Third, ionizing radiation directly damages DNA in skin cells, activating the p53 signaling pathway and increasing melanogenesis and hyperpigmentation[7]. Fourth, ionizing radiation generates free radicals in cutaneous and subcutaneous cells, damaging cellular structures and increasing melanogenesis, causing hyperpigmentation[8]. Overall, the physiological mechanisms of hyperpigmentation induced by ionizing radiation are complex and involve multiple pathways. This case suggests that a total dose of 48 Gy (39 Gy WBI + 9 Gy additional boost, 3 Gy per fraction) is associated with a risk of hyperpigmentation compared to 50 Gy WBI (2 Gy per fraction).

The strength of this case report is that the same patient was treated with different dose fractions of WBI. Even though the late skin reactions of conventional

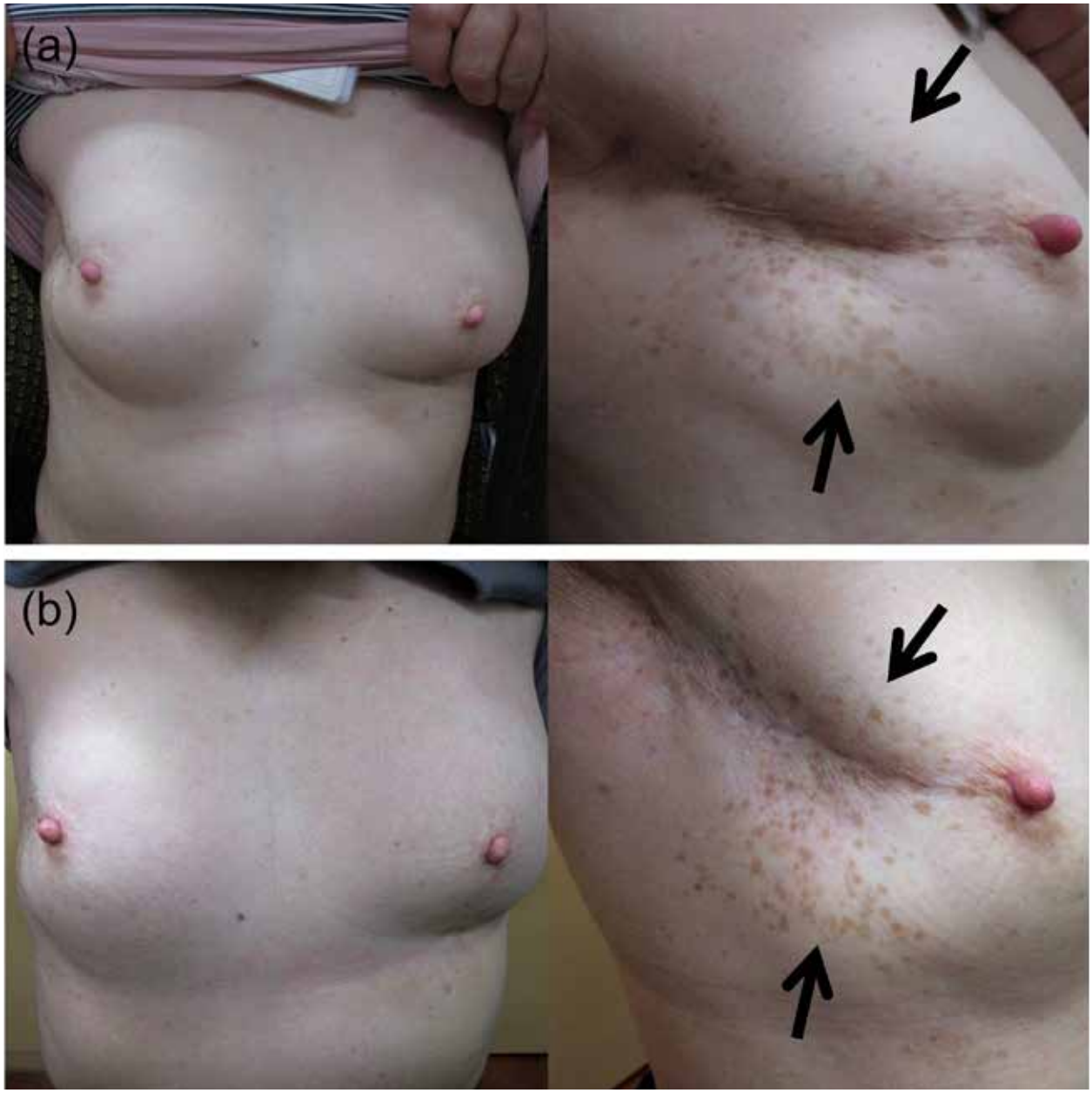


**Fig. 2:** Radiation-induced hyperpigmentation.
(a) Photographs 3 years after whole breast irradiation (WBI) and a boost. There was no hyperpigmentation in the right side of the breast that received hypofractionated WBI alone. Hyperpigmentation is seen in the outer portion of the right breast (arrows) that received an additional boost of radiotherapy.
(b) Photographs 6 years after WBI and a boost. No hyperpigmentation was observed in the areas where boost irradiation was not performed, but hyperpigmentation progressed in the area where boost irradiation was added (arrows).

and hypofractionated WBI are comparable, this case suggests that hypofractionated boost irradiation followed by hypofractionated WBI may carry a risk of hyperpigmentation. The limitation of this case report is that boost irradiation was not performed in the contralateral breast. Although a comparison could be made by adding boost irradiation to both breasts or not adding it to both, such a comparison is impossible because of the different stages, histopathologies and conditions of the postoperative margins.

## CONCLUSION

In conclusion, a single case report cannot be generalized to others without additional scientific validation. However, hypofractionated boost irradiation after hypofractionated WBI carries a risk of hyperpigmentation and should be treated with caution, even if conventional WBI did not cause late skin damage. Further studies are needed to guide the prevention of hyperpigmentation in patients receiving hypofractionated WBI and boost.

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**Authors' contribution:** All authors conceived and designed the analysis, collected the data and wrote the paper.

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Case Report

# Successful replantation of wrist level amputation on the structural, motor, and sensory aspects: Report of two patients

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## ABSTRACT

Despite the fact that there have been numerous instances of minor replantation, only a small number of reports of wrist level amputation replantation have been published. The technical specifics of two successful replantation of wrist level amputations in a 26-year-old and 36-year-old male patient, both of whom worked as carpenters for the same furniture manufacturing company using the same computerized numerical control milling machine, are reported in this case report. Both patients retained elbow and hand muscular integrity. The most important factors in achieving success are proper fixation of the amputated part in a manner that mimics the original anatomy, radical and aggressive debridement of the soft tissues, reconstruction of the defect using vein grafts, if necessary, and neural repair while maintaining proper integrity. When the requirements are met, replantation of wrist level amputations results in a superior outcome in terms of both function and appearance.

**KEY WORDS:** carpenter, functional outcomes, replantation, viability, wrist

## INTRODUCTION

Reimplantation is unquestionably far superior to all other reconstructive surgical options from both a functional and aesthetic standpoint than reattaching the amputated part to its original location. As trauma patient care has improved in many microsurgery institutions worldwide, replantation interventions have advanced as well, resulting in an almost 80 percent or more success rate overall[1].

In major amputations, there is more muscle damage in comparison to minor (distal) amputations. The more the muscle damage, the more the chance of Crush Syndrome. The major amputation injuries involve crushing syndrome, as well as main arterial and venous structure loss that can result in severe morbidity and even death, particularly during prolonged ischemia times[2]. Despite the well-established realism and functional benefits of this treatment, the majority of publications in the literature have been limited to replantation of digit amputations.

Herein, we report of two patients of replantation at the level of the wrist together with the short-term results, involving two sequential replantation resulting in successful reacquisition of both viability and function.

## CASE REPORT

### Case 1

A 26-year-old, left-hand dominant male was admitted to the emergency clinic following a milling saw injury and his left upper limb was cut at the wrist level. He was presented to our tertiary hospital 2 hours after the injury with the subtotal amputated part but without any cooling preservation. Just after the injury, the patient was initially administered to another facility, where he waited for 1 hour to transfer. At the emergency department, the patient's general condition was unstable, and he was resuscitated to stabilize vital signs. There was no additional injury to either the soft tissue of the hand or the patient.

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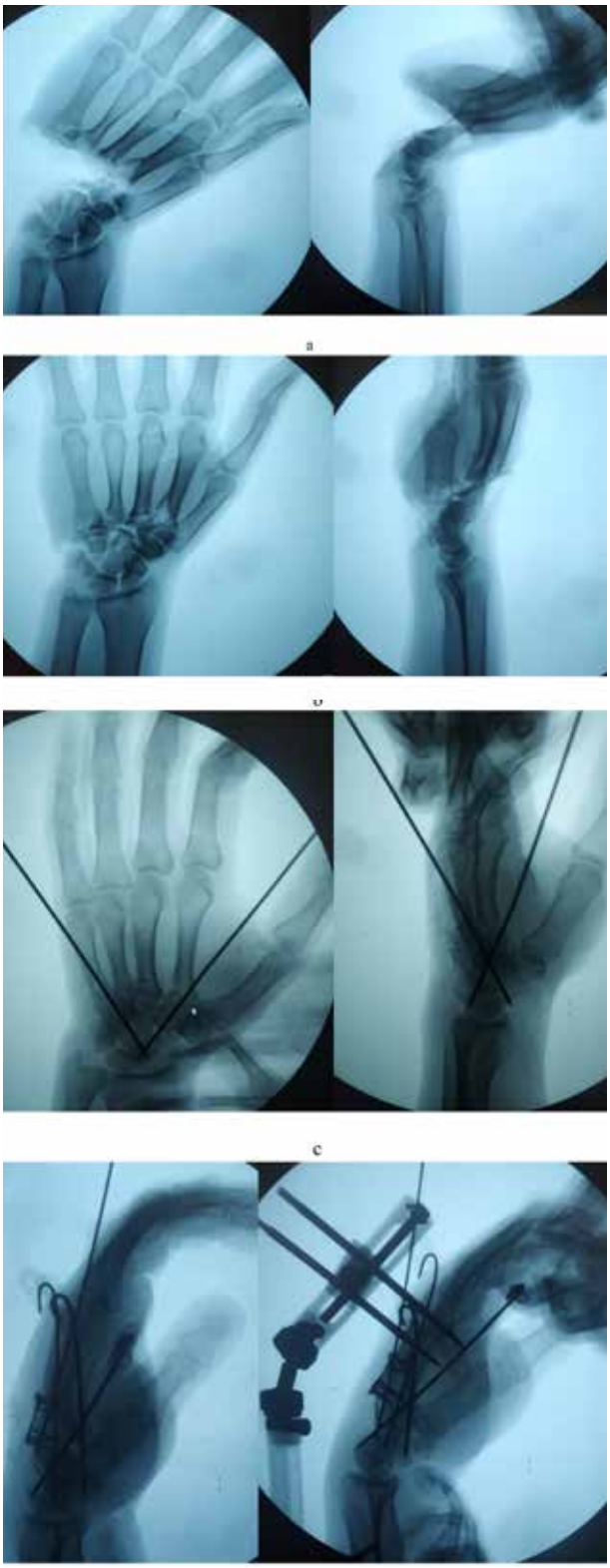

**Figure 1: Case 1:** Real time intraoperative X-ray views. **a.** Preoperative A-P view; **b.** Preoperative Lateral view; **c**. Temporary K-wire fixation A-P and lateral view just before the revascularization; **d.** Final stabilization of the wrist bones and the joint with K-wires and external fixator.

The surgery was performed under peripheral nerve block anesthesia supported with continuous intravenous sedation. The amputated part was injected with University of Wisconsin solution from the ulnar artery until clear fluid was recovered from the radial artery and vein in order to guarantee that all portions of the amputate were thoroughly clean. Recipient part of the wrist was also debrided and cleaned with saline solution (0.9% NaCl). Bone fixation was performed using K-wires and at the end of the surgery, wrist joint was fixed externally using a fixator, shown in Figure 1. Left upper limb was cut at the wrist level (Figure 2a). After anastomosis of arterial (radial and ulnar) and venous (cephalic and basilic) structures without grafting, the motor and sensory nerve anastomosis were performed. The warm ischemia time was 3 h (2 h from accident to ischemia time, 1 h during the operation) and the cold ischemia time was 1 h, yielding a total 4 h of ischemia. Since the color of the limb was normal, showing good perfusion and no edema was noted, no fasciotomy was performed (Figure 2b). One unit of erythrocyte suspension transfusion had been performed at the time of admission and again 2 units were transfused during the operation. The patient was shifted to the intensive care unit postoperatively and to the inpatient service 1 day later. Minimal elevation was applied. The patient was discharged 10 days later, and a rehabilitation program was started on the 14$^{th}$ day after the surgery. The patient is currently doing well with quite close to excellent motor and sensorial functions at the end of the 2.5 years follow-up (Figure 2c). The latest DASH score is 82.83, at the end of the 2 years follow up. Results of Semmes Weinstein monofilament test are 3.43, 3.31 and 3.94 in distal median, ulnar and radial nerve innervation regions respectively. Protective sensation is confirmed and discriminative sensation are noted as 5-9 mm in both median and ulnar nerve innervation sites. The range of motion (degree) of the wrist joint are noted at Table 1.

**Case 2**

A 36-year-old, right-hand dominant male was admitted to the emergency clinic following a milling saw (same machine as previous patient) injury and his right upper limb was cut at the wrist level (Figure 3). He was administered to the hospital just after the work-related injury without the amputated part. The amputated part was achieved one and a half hours after the injury occurred, but this time with a proper cooling preservation performed by paramedics. Similar to the pervious patient, the patient was resuscitated in the emergency department because his vital signs were unsteady. The patient and soft hand tissue were unharmed.

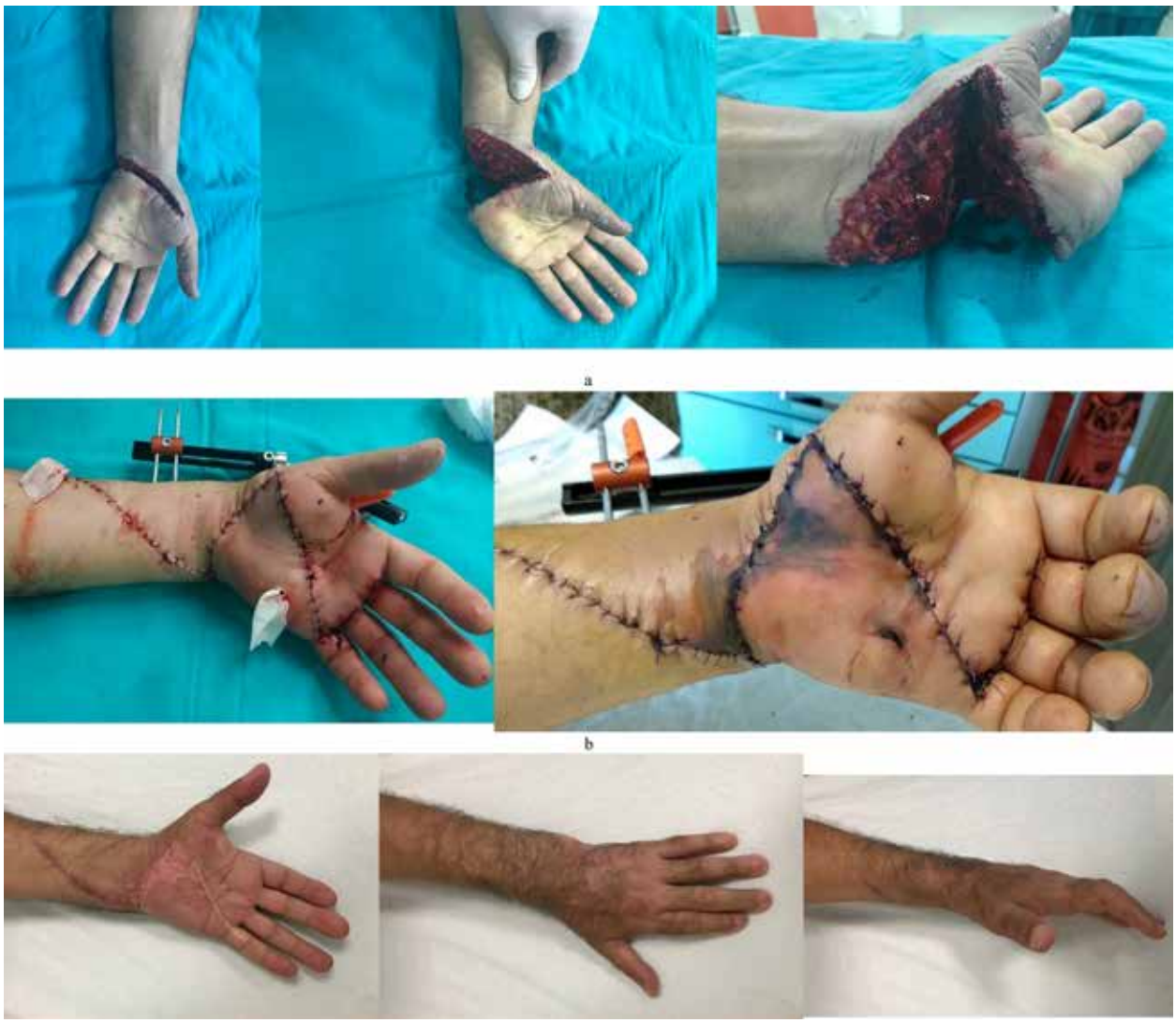

**Figure 2: Case 1:** Clinical views. **a.** Pre-operative view; **b.** Early post-operative view; **c.** Late post-operative view.

The warm ischemia time was 1.5 h (1/2 h from accident to hospital, 1 h during the operation) and the cold ischemia time was 1 h, yielding a total 2.5 h of ischemia. Just as in case 1, the fasciotomy was not performed. A total of 4 units of erythrocyte suspension were transfused, 2 units at the time of admission and 2 units during the operation. The patient was admitted to the intensive care unit postoperatively due to acute renal failure and to the inpatient service 5 days later. Early postoperative recovery was uneventful, except for 2 sessions of bedside hemodialysis. The patient was discharged 15 days later, and a rehabilitation program was started on the 21st day after the surgery. The patient continued the rehabilitation program well with quite close to good motor and sensorial functions at the end of the 6 month follow-up. The latest DASH score is 68.66, at the end of the 1 year follow up. Semmes-Weinstein monofilament test was performed and deep sensation was confirmed. Discriminative sensations were noted as 9-12 mm in both median and ulnar nerve innervation sites at the hand at the end of the 6-month follow-up. The range of motion (degree) of the wrist joint is noted in Table 1.

## DISCUSSION

The wrist joint level has been identified as the critical level for the major-minor differentiation of amputations in literature. However, owing to its anatomical formations, it cannot be classed under each of them. Anatomical structures at the level of the wrist are well organized around the tendinous processes. The vascular and neuronal structures were thinner distally than proximally. Therefore, although there are similar problems, they are distinct for large and minor replantation. In this setting, replantation must be examined anatomically rather than as major

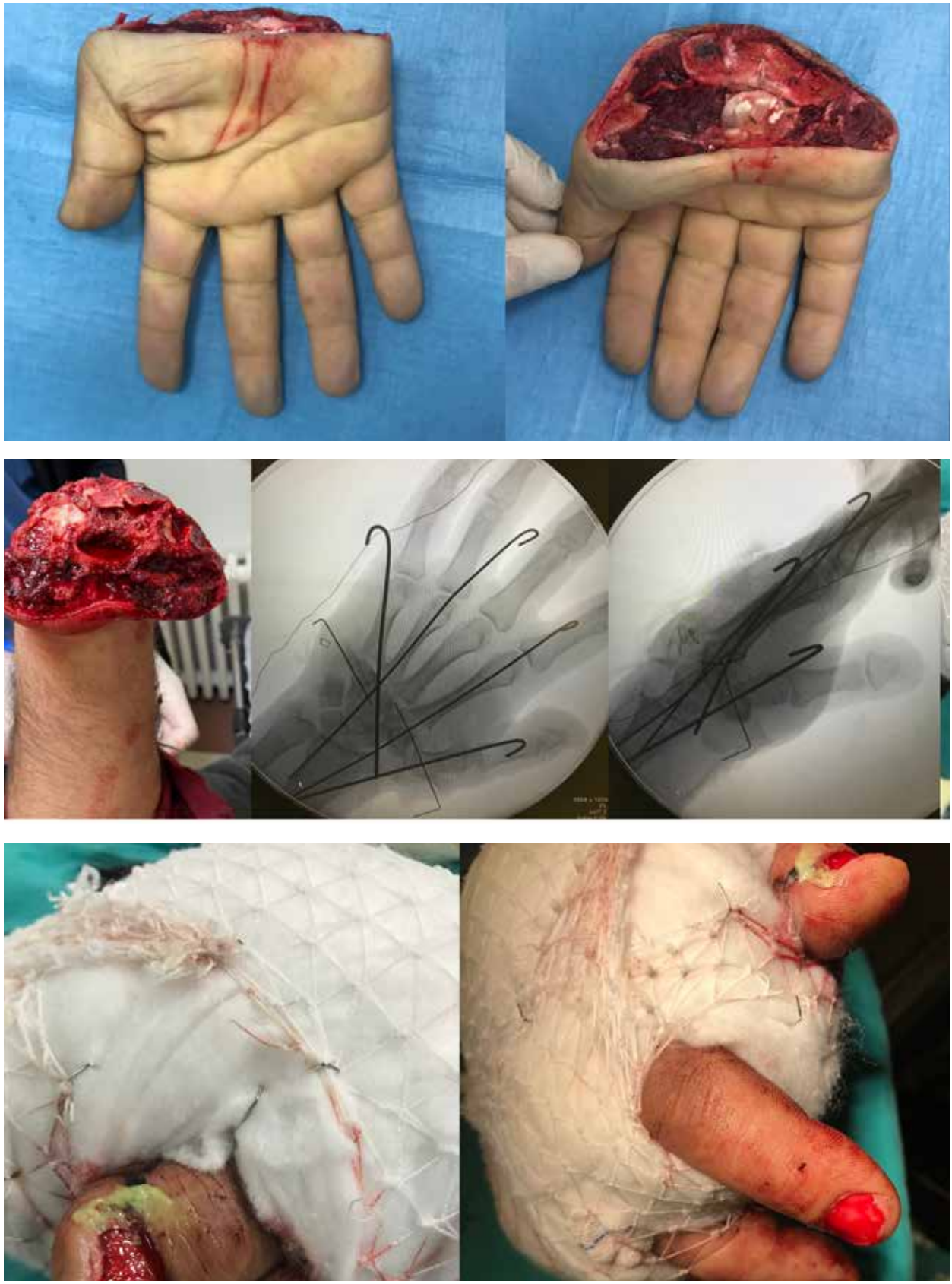

**Figure 3: Case 2:** clinical views. **a.** Pre-operative view; **b.** Intra-operative AP and Lateral view of the fixation with K-wires; **c.** Early post-operative view.

**Table 1:** Wrist range of motion values of the patients

| Wrist Range of Motion | Flexion | | Extension | | Adduction | | Abduction | |
|---|---|---|---|---|---|---|---|---|
| | Active | Passive | Active | Passive | Active | Passive | Active | Passive |
| Patient #1 | 60 | 75 | 40 | 60 | 10 | 15 | 5 | 10 |
| Patient #2 | 45 | 65 | 35 | 65 | 5 | 15 | 5 | 10 |

or minor. Amputations proximal to the distal 1/3 of the forearm and wrist joint also damage the motor endplates' muscle entrance sites[2]. Regeneration of neural structures takes longer time and is less viable. The major replantation functional results were likely to be poorer than more distal replantation due to that fact[3]. Free muscle transfers may be needed to enhance surgical outcomes[4].

One of the most important reasons for the decrease in the success of proximal 1/3 forearm amputations is excess muscle mass. Long nerve regeneration time also often causes muscle fibrosis. Proximal cutting of motor and sensory fascicules also increases the possibility of mismatching in the sensory and motor pathways during the recovery period[5].

In above-elbow replantation, the chance of nerve recovery is very low, but it must be done to preserve elbow function. If nerve regeneration fails after replantation, an under-elbow prosthesis can be used by amputation at the mid forearm level[6].

Wrist level replantation should be performed unless there is a severe crush injury. In a study, a success rate of 86% was found and all patients were satisfied with their functionality, and they stated that they would prefer replantation if they had the chance to choose again[7]. Distal forearm and wrist level replantation have been reported to have the best functional outcome[8]. The reason is that the neuromuscular junction that provides the functions of the hand is intact. The problem at this level is tendon healing and adhesion. Due to the level of these injuries, the time and distance for nerve regeneration is short. Therefore, target motor and sensory endplates have more chances for early re-innervation and return[6-7].

There are different surgical difficulties in wrist level replantation. In order to obtain optimum results in this location, it is essential not to disturb the perfusion of the forearm muscles, to protect the primary optimal repair of the tendons, and to protect the muscle nerve motor units for the hand[9]. Some of the advantages of wrist level replantation are the large vessel and nerve structure, easy stabilization of the bone structure, the presence of only tendon, bone and ligament, the absence of muscle structure, and the low risk of infection and metabolic disease. Motor recovery is better as the forearm muscles are intact and there is a short distance for regeneration of the repaired nerves[10].

## CONCLUSION

For a successful replantation of wrist level amputation on the structural, motor and sensory aspect, it is critical to have a timely, correct organization with no delays in any step of the preparation process or throughout the surgical procedure. It should also be emphasized that the most essential variables in achieving excellent end results are the fundamental needs, which may be characterized as the motivation, compliance, and socioeconomic level of the patient, as well as the experience of the reconstructive microsurgeon. When these conditions are met, replantation of wrist level amputations result in a superior outcome in terms of both function and appearance.

## ACKNOWLEDGMENT

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**Ethical Approval:** This article does not contain any study with human participants or animals performed by any of the authors.

**Informed Consent:** The authors certify that they have obtained all appropriate patient consent forms. The patients and/or their families were informed that data from the case would be submitted for publication and gave their consent. The patient understands that his name and initials will not be published and due efforts will be made to conceal their identity; however, anonymity cannot be guaranteed.

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# Selected Abstracts of Articles Published Elsewhere by Authors in Kuwait

Kuwait Medical Journal 2026; 58 (1): 62 - 64

## Vitamin D status during pregnancy modulates the effect of pre-pregnancy obesity on gestational diabetes mellitus risk: a birth cohort study

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**Public Health Nutr. 2026 Jan 26:1-30. doi: 10.1017/S1368980026101906. Online ahead of print.**

**OBJECTIVE**
To determine whether gestational vitamin D status modulates the effect of pre-pregnancy obesity on gestational diabetes mellitus (GDM) risk while stratifying by maternal age.

**DESIGN**
Birth cohort.

**SETTING**
A major maternity hospital in Kuwait.

**PARTICIPANTS**
Pregnant women in their second/third trimester of gestation were enrolled. Pre-pregnancy body mass index (BMI, kg/m$^2$) was categorized as under/normal weight (<25.0), overweight (25.0 to <30.0), and obesity (≥30.0). Gestational 25-hydroxyvitamin D concentrations were categorized as deficiency (<50 nmol/L) or insufficiency/sufficiency (≥50 nmol/L). GDM status was ascertained according to international guidelines. Adjusted odds ratios (aOR) and 95% confidence intervals (CI) were estimated using logistic regression.

**RESULTS**
Data from 957 pregnant women were analyzed, with GDM affecting 166 (17.4%) pregnancies. Pre-pregnancy obesity and gestational vitamin D deficiency was ascertained in 275 (28.7%) and 533 (55.7%) pregnant women, respectively. The association between pre-pregnancy obesity and GDM risk differed according to maternal age and gestational vitamin D status ($P_{\text{interaction[BMI × age × vitamin D]}}$=0.041). Among women aged <35 years (n=710), pre-pregnancy obesity compared to under/normal weight was associated with increased GDM risk among women with gestational vitamin D deficiency (aOR: 2.72, 95% CI: 1.18-6.23) and vitamin D insufficiency/sufficiency (2.55, 1.15-5.62). In contrast, among women aged ≥35 years (n=247), pre-pregnancy obesity compared to under/normal weight was associated with increased GDM risk among women with gestational vitamin D deficiency (6.92, 1.45-33.04), but not among women with vitamin D insufficiency/sufficiency (1.13, 0.36-3.56).

**CONCLUSIONS**
Gestational vitamin D status modulates the effect of pre-pregnancy obesity on GDM risk in an age-specific manner.

# A comparative study of dermatology education in high and low prevalence areas: Kuwait University and the University of Aberdeen

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**BMC Med Educ. 2026 Jan 21. doi: 10.1186/s12909-026-08615-y. Online ahead of print.**

**BACKGROUND**
Dermatological disease prevalence differs across regions, which may influence how medical schools prioritise dermatology training. Skin cancer predominates in Scotland, while vitiligo is more common in Kuwait. This study compares dermatology curricula at Kuwait University (KU) and the University of Aberdeen (UoA) to examine how local disease prevalence shapes educational focus and student preparedness.

**OBJECTIVES**
To evaluate differences in curriculum content, student confidence, and clinical exposure between KU and UoA, and to assess the global relevance of their dermatology teaching.

**METHODS**
A mixed-methods comparative design was used, combining curriculum document analysis, online surveys of final-year medical students, and semi-structured interviews with faculty and residents. Quantitative data assessed knowledge and confidence, while qualitative themes examined adequacy and exposure.

**RESULTS**
Both curricula reflected local disease patterns: KU emphasised pigmentary disorders such as vitiligo, while UoA focused on malignant melanoma (MM) and other skin cancers. KU students reported greater confidence with autoimmune and pigmentary conditions, whereas UoA students demonstrated more substantial knowledge of MM and eczema. Both cohorts and faculty identified insufficient dermatology exposure overall.

**CONCLUSIONS**
Dermatology education at KU and UoA aligns with local prevalence but may limit preparedness for managing conditions common elsewhere. Expanding international electives, case-based learning, and global curricular integration may enhance dermatological competency across regions.

# The relation of corneal arcus with cardiovascular diseases: a systematic review

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[3]Kuwait Board of Ophthalmology, Kuwait Institute for Medical Specialization, Kuwait City, KWT.
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Cureus. 2025 Dec 19;17(12):e99615. doi: 10.7759/cureus.99615. eCollection 2025 Dec.

The prognostic value of corneal arcus (arcus senilis) for cardiovascular disease (CVD) remains debated. We evaluated associations of arcus with cardiometabolic risk, prevalent CVD, and incident events, and

summarized how consistently studies adjusted for standard risk factors. We systematically reviewed observational studies (1960-2017) from Asia, Europe, and North America. Designs included cross-sectional, case-control, and prospective cohorts. Risk of bias was appraised using the Newcastle-Ottawa Scale (cohort/case-control) and the Joanna Briggs Institute checklist (cross-sectional). Twelve studies met the inclusion criteria. The prevalence of arcus increased with age and was higher in men. Cross-sectional and case-control evidence showed consistent associations with atherogenic lipid profiles and a higher burden of prevalent CVD. Prospective findings were mixed: arcus predicted incident events in targeted subgroups (notably younger men and some Asian male populations) but offered limited independent prognostic value and minimal incremental discrimination in general, older populations once age, sex, and lipids were considered. Overall risk of bias was low in most cohorts; moderate ratings reflected limited confounder control, non-slit-lamp exposure assessment, or sampling constraints. Corneal arcus appears to primarily reflect cumulative lipid exposure. In adults under 50 years or select higher-risk men, its presence should prompt lipid evaluation and risk review; however, its low sensitivity means the absence of arcus does not exclude CVD.

## Age at first marriage, menopause status and cervical cancer risk in a middle eastern country: a national cancer registry-based case-control study

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**Cancer Causes Control. 2026 Jan 17;37(2):18. doi: 10.1007/s10552-025-02087-z.**

**BACKGROUND**

Cervical cancer ranks as the fourth leading cause of cancer-related morbidity and mortality among women worldwide. Despite its global significance, evidence on cervical cancer risk factors in Kuwait remains limited. This case-control study aimed to identify factors associated with cervical cancer among women in Kuwait.

**METHODS**

A total of 50 cervical cancer cases were recruited from the Kuwait Cancer Control Center, and 155 controls were selected from public-sector employees in a 1:3 ratio. Data were collected using a structured questionnaire. Adjusted odds ratios ($OR_{adj}$) and 95% confidence intervals (CI) were estimated through multivariable logistic regression analysis.

**RESULTS**

Compared with controls, cases were more likely to have first marriage at a younger age (< 25 vs. ≥ 25 years) ($OR_{adj}$ = 5.52; 95% CI: 1.34-22.82, p = 0.018), to be unaware of HPV vaccine availability ($OR_{adj}$ = 7.63; 95% CI: 1.60-36.39, p = 0.011) or tended to be in menopause ($OR_{adj}$ = 5.17; 95% CI: 1.64-16.33, p = 0.005). These associations were adjusted for the smoking status (ever vs. never).

**CONCLUSION**

Younger age at first marriage, being in menopause, and unawareness of HPV vaccine availability were independently associated with an increased risk of cervical cancer. These findings should be regarded as preliminary and hypothesis-generating, offering a foundation for further research on this important women's health issue in Kuwait and comparable settings. Larger studies are warranted to confirm these results and to identify additional determinants of cervical cancer risk.

# Forthcoming Conferences and Meetings

Compiled and edited by
**Vineetha Elizabeth Mammen**

Kuwait Medical Journal 2026; 58 (1): 65 - 73

International Conference on **Medical, Biological and Pharmaceutical Sciences**
*South Korea*, Seoul
Mar 01, 2026
Organized by: EASRD
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International Conference on **Medical and Biosciences**
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Mar 01, 2026
Organized by: Research world
Email-Id: info@researchworld.org

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Mar 01, 2026
Organized by: Academicsera
Email-Id: info@academicsera.com

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Mar 03, 2026
Organized by: Global Conference
Email-Id: summit.globalconference@gmail.com

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Organized by: Theires
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Mar 05, 2026
Organized by: Research world
Email-Id: info@researchworld.org

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Organized by: Conference Research Network
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Organized by: Biofora
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Organized by: Universal Research Cluster
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# WHO-Facts Sheet

**1. Dengue**
**2. Food additives**
**3. Ionizing radiation and health effects**
**4. Meningitis**
**5. Post COVID-19 condition (long COVID)**

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## 1. Dengue

**KEY FACTS**

- Dengue is a viral infection caused by the dengue virus (DENV), which is transmitted to humans through the bite of infected mosquitoes.
- About half of the world's population is now at risk of dengue, with an estimated 100–400 million infections occurring each year.
- Dengue is found in tropical and sub-tropical climates worldwide, mostly in urban and semi-urban areas.
- While many DENV infections are asymptomatic or produce only mild illness, DENV can occasionally cause more severe cases, and even death.
- Prevention and control of dengue rely on vector control. There is no specific treatment for dengue/severe dengue, and early detection and access to proper medical care greatly lower fatality rates of severe dengue.

**Overview**

Dengue (break-bone fever) is a viral infection that is spread from mosquitoes to people. It is more common in tropical and subtropical than in temperate climates.

Most people who get dengue do not have symptoms. For those who do, the most common symptoms are high fever, headache, body aches, nausea and rash. Most get better in 1–2 weeks. Some develop severe dengue and need care in a hospital.

In severe cases, dengue can be fatal. You can lower your risk of dengue by avoiding mosquito bites, especially during the day.

Dengue is treated through pain management as there is no specific treatment currently.

**Symptoms**

Most people with dengue have mild or no symptoms and will get better in 1–2 weeks. Rarely, dengue can be severe and lead to death.

If symptoms occur, they usually begin 4–10 days after infection and last for 2–7 days. Symptoms may include:

- high fever (40°C/104°F)
- severe headache
- pain behind the eyes
- muscle and joint pains
- nausea
- vomiting
- swollen glands
- rash.

Individuals who are infected for the second time are at greater risk of severe dengue. The symptoms of severe dengue often come after the fever has gone away and may include:

- severe abdominal pain
- persistent vomiting
- rapid breathing
- bleeding gums or nose
- fatigue
- restlessness
- blood in vomit or stool
- being very thirsty
- pale and cold skin
- feeling weak.

People with these severe symptoms should seek care immediately. After recovery, people who have had dengue may experience fatigue for several weeks.

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**Diagnostics and treatment**

Laboratory-based and point of care diagnostics are critical to control and manage dengue, yet global disparities in laboratory capabilities present significant challenges. The diagnostic algorithms, testing strategies and test methodologies employed vary, depending on the capabilities of national laboratory systems. The wide range of available tests – including nucleic acid amplification tests (NAATs), enzyme-linked immunosorbent assays (ELISAs) and rapid diagnostic tests (RDTs) – vary significantly in quality and performance.

Laboratory testing for arboviruses can be accomplished through either direct detection methods such as virus isolation, molecular detection of nucleic acid or antigen testing, including rapid diagnostic tests (RDTs) within the first week of illness.

There is no specific treatment for dengue, although pain can be managed with medication such as paracetamol (acetaminophen). Non-steroidal anti-inflammatory medicines such as ibuprofen and aspirin should be avoided as they can increase the risk of bleeding. For people with severe dengue, hospitalization is often necessary.

**Global burden**

The incidence of dengue has grown dramatically worldwide in recent decades, with the number of cases reported to WHO increasing from 505 430 cases in 2000 to 14.6 million in 2024. The vast majority of cases are asymptomatic or mild and self-managed, and hence the actual numbers of dengue cases are under-reported. The disease is now endemic in more than 100 countries.

In 2024, more cases of dengue were recorded than ever before in a 12-month period, affecting over 100 countries on all continents. During 2024, ongoing transmission, combined with an unexpected spike in dengue cases, resulted in a historic high of over 14.6 million cases and more than 12 000 dengue-related deaths reported. The Region of the Americas contributed a significant proportion of the global burden, with over 13 million cases reported to WHO.

Several factors are associated with the increasing risk of spread of the dengue epidemic, including the changing distribution of the responsible vectors (chiefly *Aedes aegypti and Aedes albopictus*), especially in previously dengue-naive countries; climate change leading to increasing temperatures, high rainfall and humidity; fragile and overburdened health systems; limitations in surveillance and reporting; and political and financial instabilities in countries facing complex humanitarian crises and high population movements.

One modelling estimate indicates 390 million dengue virus infections per year, of which 96 million manifest clinically(1).A recent study on the prevalence of dengue estimates that 5.6 billion people are at risk of infection with dengue and other arboviruses(2).

From January to July 2025, over 4 million cases and over 3000 deaths have been reported to WHO from 97 countries. Dengue is spreading to new areas, including the European and Eastern Mediterranean regions. In 2024, 308 cases were reported to WHO from three European countries (France, Italy and Spain) and an additional 1291 cases and four deaths were recorded in the French overseas territories of Mayotte and Réunion.

**Transmission**

**Transmission through the mosquito bite**

The dengue virus is transmitted to humans through the bites of infected female mosquitoes, primarily the *Aedes aegypti* mosquito. Other species within the *Aedes* genus can also act as vectors, but their contribution is normally secondary to *Aedes aegypti*.

After feeding on a DENV-infected person, the virus replicates in the mosquito midgut before disseminating to secondary tissues, including the salivary glands. The time it takes from ingesting the virus to actual transmission to a new host is termed the extrinsic incubation period (EIP). The EIP takes about 8–12 days when the ambient temperature is 25–28°C. Variations in the EIP are not only influenced by ambient temperature but also by several other factors – such as the magnitude of daily temperature fluctuations, the virus genotype, and the initial viral concentration – which can also alter the time it takes for a mosquito to transmit the virus. Once infectious, a mosquito can transmit the virus for the rest of its life.

**Human-to-mosquito transmission**

Mosquitoes can become infected by people who are viremic with DENV. This can be someone who has a symptomatic dengue infection, someone who is yet to have a symptomatic infection (those who are pre-symptomatic), and also someone who shows no signs of illness (those who are asymptomatic).

Human-to-mosquito transmission can occur up to 2 days before someone shows symptoms of the illness, and up to 2 days after the fever has resolved.

The risk of mosquito infection is positively associated with high viremia and high fever in the patient; conversely, high levels of DENV-specific antibodies are associated with a decreased risk of mosquito infection. Most people are viremic for about 4–5 days, but viremia can last as long as 12 days.

**Maternal transmission**

The primary mode of transmission of the DENV between humans involves mosquito vectors. There is evidence, however, of the possibility of maternal

transmission (i.e. from a pregnant mother to her baby). At the same time, vertical transmission rates appear low, with the risk of vertical transmission seemingly linked to the timing of acquiring the dengue infection during pregnancy. When a mother does have a dengue infection when she is pregnant, babies may suffer from pre-term birth, low birthweight and fetal distress.

**Other transmission modes**

Rare cases of transmission via blood products, organ donation and transfusions have been recorded. Similarly, transovarial transmission of the virus within mosquitoes has also been recorded.

**Risk factors**

Previous infection with DENV increases the risk of an individual developing severe dengue. Urbanization (especially rapid, unplanned urbanization), is associated with dengue transmission through multiple social and environmental factors: population density, human mobility, access to reliable water source, water storage practices, etc.

Community risks to dengue also depend on population knowledge, attitudes and practices towards dengue, as exposure is closely related to behaviours such as water storage, plant-keeping and self-protection against mosquito bites. Routine vector surveillance and control activities and targeted community engagement greatly enhance resilience.

Vectors can adapt to new environments and climate. The interaction between the dengue virus, the host and the environment is dynamic. Consequently, disease risks may change and shift with climate change in tropical and subtropical areas, in combination with increased urbanization and population movement.

**Prevention and control**

The mosquitoes that spread dengue are active during the day.

To lower your risk of getting dengue, protect yourself from mosquito bites by using:

- clothes that cover as much of your body as possible;
- mosquito nets, ideally sprayed with insect repellent, if sleeping during the day;
- window screens;
- mosquito repellents (containing DEET, Picaridin or IR3535); and
- coils and vaporizers.

To prevent mosquitoes from breeding:

- implement environmental management and modification practices to stop mosquitoes from accessing egg-laying habitats;
- dispose of solid waste properly and remove artificial habitats that can hold water;
- cover, empty and clean domestic water storage containers on a weekly basis; and
- apply appropriate insecticides to water storage outdoor containers.

If you get dengue, it's important to:

- rest;
- drink plenty of liquids;
- use acetaminophen (paracetamol) for pain;
- avoid non-steroidal anti-inflammatory medication such as ibuprofen and aspirin; and
- watch for severe symptoms and contact your doctor as soon as possible if you notice any.

Currently, one vaccine (QDenga) is available and licensed in some countries. However, it is recommended only for those aged 6–16 years in high transmission settings. Several additional vaccines are under evaluation.

**WHO response**

WHO responds to dengue by:

- supporting countries in the confirmation of outbreaks through its collaborating network of laboratories;
- providing technical advice and guidance to countries for the effective management of dengue outbreaks;
- supporting countries to improve their reporting systems and capture the true burden of the disease;
- providing training on clinical management, diagnosis and vector control at the country and regional levels in collaboration with its collaborating centres;
- formulating evidence-based strategies and policies;
- supporting countries to develop dengue prevention and control strategies and adopt the Global Vector Control Response (2017–2030) and the Global Arbovirus Initiative (2022–2025);
- reviewing and making recommendations on the development of new tools, including insecticide products and application technologies;
- gathering official records of dengue and severe dengue from over 100 Member States; and
- publishing guidance and handbooks for surveillance, case management, diagnosis, dengue prevention and control for Member States.

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## 2. Food additives

**KEY FACTS**

- Food additives are substances primarily added to processed foods, or other foods produced on an industrial scale, for technical purposes, e.g. to improve safety, increase the amount of time a food can be stored, or modify sensory properties of food.
- Food additives are substances not normally consumed as a food by themselves and not normally used as typical ingredients in foods. Most minimally processed and unprocessed foods do not contain food additives.
- Food additives are assessed for potential harmful effects on human health before they are approved for use.
- Authoritative bodies at the national, regional and international levels are responsible for evaluating the safety of food additives.
- The Joint FAO/WHO Expert Committee on Food Additives (JECFA) is the international body responsible for evaluating the safety of food additives for use in foods that are traded internationally.

**Overview**

Many different food additives have been developed over time to meet the needs of large-scale food processing. Additives are added to ensure processed food remains safe and in good condition throughout its journey from factories or industrial kitchens, to warehouses and shops, and finally to consumers. Additives are also used to modify the sensory properties of foods including taste, smell, texture and appearance.

Food additives can be derived from plants, animals or minerals, or they can be chemically synthesized. There are several thousand food additives used, all of which are designed to do a specific job. Food additives can be grouped into 3 broad categories based on their function.

**Flavouring agents**

Flavouring agents are chemicals that impart flavours or fragrances and are added to food to modify its aroma or taste. They are the most common type of additive used in foods, with hundreds of varieties used in a wide variety of foods, from confectionery and soft drinks to cereal, cake and yoghurt. Flavouring agents can be extracted from naturally occurring sources (e.g. plant or animal sources) or chemically synthesized. Flavours extracted directly from naturally occurring sources are often referred to as natural flavours. Such flavours can also be chemically synthesized and are sometimes referred to as nature made or nature identical to indicate that although the flavour molecule itself is naturally occurring it hasn't been extracted from its source, but synthesized to be identical. Artificial flavouring agents are chemicals that do not exist in nature but are synthesized to imitate natural flavours or elicit other taste sensations. Culinary ingredients, including spices, nuts and dried fruits or vegetables, can also modify aroma or taste, but are generally not considered flavouring agents.

**Enzyme preparations**

Enzyme preparations are a type of additive that may or may not end up in the final food product. Enzymes are naturally occurring proteins that boost biochemical reactions by breaking down larger molecules into their smaller building blocks. They can be obtained by extraction from plants or animal products or from micro-organisms such as bacteria and are used as alternatives to chemical-based technology. They are mainly used in baking (to improve the dough), for manufacturing fruit juices (to increase yields), in wine making and brewing (to improve fermentation), as well as in cheese manufacturing (to improve curd formation).

**Other additives**

Other food additives are used for a variety of reasons, such as preservation, colouring and sweetening. They are added when food is prepared, packaged, transported, or stored, and they eventually become a component of the food.

Preservatives can slow decomposition caused by mould, air, bacteria or yeast. In addition to maintaining the quality of the food, preservatives help control contamination that can cause foodborne illness, including life-threatening botulism.

Colouring is added to food to replace colours lost during processing or other production, or to make food appear more attractive.

Non-sugar sweeteners are often used as an alternative to sugar because they contribute fewer or no calories when added to food. WHO has issued a recommendation against the use of non-sugar sweeteners in general, based on evidence that they don't seem to benefit long term weight loss or maintenance and may increase risk of noncommunicable diseases.

**Safety assessments**

Food additives are assessed for potential harmful effects on human health before they are approved for use. Authoritative bodies at the national, regional and international levels are responsible for evaluating the safety of food additives. The Joint FAO/WHO

Expert Committee on Food Additives (JECFA) is the international body responsible for evaluating the safety of food additives for use in foods that are traded internationally.

**WHO response**

**Evaluating the health risk of food additives**

WHO, in cooperation with the Food and Agriculture Organization of the United Nations (FAO), is responsible for assessing the risks to human health from food additives. Risk assessments of food additives are conducted by an independent, international expert scientific group – the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

Only food additives that have undergone a JECFA safety assessment and are found not to present an appreciable health risk to consumers can be used internationally. This applies whether food additives come from natural sources or they are synthetic. National authorities, either based on the JECFA assessment or a national assessment, can then authorize the use of food additives at specified levels for specific foods.

JECFA evaluations are based on scientific reviews of all available biochemical, toxicological, and other relevant data on a given additive – mandatory tests in animals, research studies and observations in humans are considered. The toxicological tests required by JECFA include acute, short-term and long-term studies that determine how the food additive is absorbed, distributed and excreted, and possible harmful effects of the additive or its by-products at certain exposure levels.

The starting point for determining whether a food additive can be used without having harmful effects is to establish the acceptable daily intake (ADI). The ADI is an estimate of the amount of an additive in food or drinking water that can be safely consumed daily over a lifetime without adverse health effects.

**International standards for the safe use of food additives**

The safety assessments completed by JECFA are used by the joint intergovernmental food standard-setting body of FAO and WHO, the Codex Alimentarius Commission, to establish levels for maximum use of additives in food and drinks. Codex standards are the reference for national standards for consumer protection, and for the international trade in food, so that consumers everywhere can be confident that the food they eat meets the agreed standards for safety and quality, no matter where it was produced.

Once a food additive has been found to be safe for use by JECFA and maximum use levels have been established in the Codex General Standard for Food Additives, national food regulations need to be implemented permitting the actual use of a food additive.

**How do I know which additives are in my food?**

The Codex Alimentarius Commission also establishes standards and guidelines on food labelling. These standards are implemented in most countries, and food manufacturers are obliged to indicate which additives are in their products. In the European Union, for example, there is legislation governing labelling of food additives according to a set of pre-defined E-numbers. People who have allergies or sensitivities to certain food additives should check labels carefully.

WHO encourages national authorities to monitor and ensure that food additives in food and drinks produced in their countries comply with permitted uses, conditions and legislation. National authorities should oversee the food business, which carries the primary responsibility for ensuring that the use of a food additive is safe and complies with legislation.

## 3. Ionizing radiation and health effects

**KEY FACTS**

- Ionizing radiation is a type of energy released by atoms in the form of electromagnetic waves or particles.
- People are exposed to natural sources of ionizing radiation, such as in soil, water, and vegetation, as well as in human-made sources, such as x-rays in medical devices.
- Ionizing radiation has many beneficial applications, including uses in medicine, industry, agriculture and research.
- As the use of ionizing radiation increases, so does the potential for health hazards if not properly used or contained.
- Acute health effects such as skin burns or acute radiation syndrome can occur when doses of radiation exceed very high levels.
- Low doses of ionizing radiation can increase the risk of longer term effects such as cancer.

**What is ionizing radiation?**

Ionizing radiation is a type of energy released by atoms that travels in the form of electromagnetic waves (gamma or X-rays) or particles (neutrons, beta or alpha). The spontaneous disintegration of atoms is called radioactivity, and the excess energy emitted is a form of ionizing radiation. Unstable elements which disintegrate and emit ionizing radiation are called radionuclides.

All radionuclides are uniquely identified by the type of radiation they emit, the energy of the radiation, and their half-life.

The activity — used as a measure of the amount of a radionuclide present — is expressed in a unit called the becquerel (Bq): one becquerel is one disintegration per second. The half-life is the time required for the activity of a radionuclide to decrease by decay to half of its initial value. The half-life of a radioactive element is the time that it takes for one half of its atoms to disintegrate. This can range from a mere fraction of a second to millions of years (e.g. iodine-131 has a half-life of 8 days while carbon-14 has a half-life of 5730 years).

**Radiation sources**

People are exposed to natural radiation sources as well as human-made sources on a daily basis. Natural radiation comes from many sources including more than 60 naturally-occurring radioactive materials found in soil, water and air. Radon, a naturally-occurring gas, emanates from rock and soil and is the main source of natural radiation. Every day, people inhale and ingest radionuclides from air, food and water.

People are also exposed to natural radiation from cosmic rays, particularly at high altitude. On average, 80% of the annual dose of background radiation that a person receives is due to naturally occurring terrestrial and cosmic radiation sources. Background radiation levels vary geographically due to geological differences. Exposure in certain areas can be more than 200 times higher than the global average.

Exposure to radiation also comes from human-made sources ranging from nuclear power generation to medical uses of radiation for diagnosis or treatment. Today, the most common human-made sources of ionizing radiation are medical devices, including x-ray machines and Computed Tomography (CT) scanners.

**Exposure to ionizing radiation**

People can be exposed to ionizing radiation under different circumstances, at home or in public places (public exposures), at their workplaces (occupational exposures), or in a medical setting (medical exposures).

Exposure to radiation may occur through internal or external pathways. Internal exposure to ionizing radiation occurs when a radionuclide is inhaled, ingested or otherwise enters into the bloodstream (for example, by injection or through wounds). Internal exposure stops when the radionuclide is eliminated from the body, either spontaneously (such as through excreta) or as a result of a treatment.

External exposure may occur when airborne radioactive material (such as dust, liquid, or aerosols) is deposited on skin or clothes. This type of radioactive material can often be removed from the body by washing. Exposure to ionizing radiation can also result from irradiation from an external source, such as medical radiation exposure from x-rays. External irradiation stops when the radiation source is shielded or when the person moves outside the radiation field.

Exposure to ionizing radiation can be classified for radiation protection purposes into three exposure situations, i.e. planned, existing and emergency situations. Planned exposure situations result from the deliberate introduction and operation of radiation sources with specific purposes, as is the case with the medical use of radiation for diagnosis or treatment of patients, or the use of radiation in industry or research. Existing exposure occurs where radiation already exists and a decision on control must be taken – for example, exposure to radon in homes or workplaces or exposure to natural background radiation from the environment. Emergency exposure situations result from unexpected events requiring prompt response, such as nuclear accidents or malicious acts.

Medical use of radiation accounts for 98 % of the population dose contribution from all human-made sources, and represents 20% of the total population exposure. Annually worldwide, more than 4200 million diagnostic radiology examinations are performed, 40 million nuclear medicine procedures are carried out, and 8.5 million radiotherapy treatments are given.

**Health effects of ionizing radiation**

Radiation damage to tissue and/or organs depends on the dose of radiation received, or the absorbed dose which is expressed in a unit called the gray (Gy). The potential damage from an absorbed dose depends on the type of radiation and the sensitivity of the different tissues and organs.

The effective dose is used to measure ionizing radiation in terms of the potential for causing harm. The sievert (Sv) is the unit of effective dose that takes into account the type of radiation and sensitivity of tissues and organs. It is a way to measure ionizing radiation in terms of the potential for causing harm. In addition to the amount of radiation (dose), the rate at which the dose is delivered (dose rate), described in microsieverts per hour (μSv/hour) or millisievert per year (mSv/year), is an important parameter.

Beyond certain thresholds, radiation can impair the functioning of tissues and/or organs and can produce acute effects such as skin redness, hair loss, radiation burns, or acute radiation syndrome. These effects are more severe at higher doses and higher dose rates. For instance, the dose threshold for acute radiation syndrome is about 1 Sv (1000 mSv).

If the radiation dose is low and/or it is delivered over a long period of time (low dose rate), the risk is

substantially low because there is a greater likelihood of repairing the damage. There is still a risk of long-term effects such as cataract or cancer, however, that may appear years or even decades later. Effects of this type will not always occur, but their likelihood is proportional to the radiation dose. This risk is higher for children and adolescents as they are significantly more sensitive to radiation exposure than adults.

Epidemiological studies on populations exposed to radiation, such as the survivors of the atomic bombings or radiotherapy patients, showed a significant increase of cancer risk at doses above 100 mSv. More recently, some epidemiological studies in individuals exposed to medical exposure during childhood (paediatric CT) have suggested that cancer risk may increase even at lower doses (between 50-100 mSv).

Prenatal exposure to ionizing radiation may induce brain damage in foetuses following an acute dose exceeding 100 mSv between weeks 8-15 of pregnancy and 200 mSv between weeks 16-25 of pregnancy. Before week 8 or after week 25 of pregnancy human studies have not shown radiation risk to fetal brain development. Epidemiological studies indicate that the cancer risk after fetal exposure to radiation is similar to the risk after exposure in early childhood.

**WHO response**

WHO works to strengthen radiation protection of patients, workers and the public worldwide. It provides Member States with evidence-based guidance, tools and technical advice on public health issues related to ionizing radiation. Focusing on public health aspects of radiation protection, WHO covers activities related to radiation risk assessment, management and communication.

In line with its core function on "setting norms and standards and promoting and monitoring their implementation", WHO contributed to the development, co-sponsored and endorsed the latest version of the International Basic Safety Standards (BSS) with 7 other international organizations, and is currently working to support the implementation of the BSS in its Member States.

## 4. Meningitis

**KEY FACTS**

- Meningitis is a devastating disease that can be deadly and often results in serious long-term health issues.
- Meningitis remains a major global public health challenge.
- Many organisms can cause meningitis, including bacteria, viruses, fungi and parasites.
- Bacterial meningitis is of particular concern. Around 1 in 6 people who get this type of meningitis die and 1 in 5 have severe complications.
- Epidemics of meningitis are seen across the world, particularly in sub-Saharan Africa.
- Vaccines are the most effective way to deliver long-lasting protection.

**Overview**

Meningitis is the inflammation of the tissues surrounding the brain and spinal cord. It can be infectious or non-infectious in origin, can be associated with high risk of death and long-term complications, and requires urgent medical care.

Meningitis remains a significant global health threat. It can be caused by several species of bacteria, viruses, fungi and parasites. Injuries, cancers and drugs cause a small number of cases.

Bacterial meningitis is the most serious type of meningitis. It is a severe, life-threatening condition that can often lead to long-term adverse health consequences. There are four main causes of acute bacterial meningitis:

- *Neisseria meningitidis* (meningococcus)
- *Streptococcus pneumoniae* (pneumococcus)
- *Haemophilus influenzae*
- *Streptococcus agalactiae* (group B streptococcus).

These bacteria are responsible for more than half of the deaths from meningitis globally and can cause other severe diseases like sepsis and pneumonia.

Additional important causes of meningitis worldwide include other bacteria species (e.g. *Mycobacterium tuberculosis*, non-typhoidal *Salmonella spp*, Listeria monocytogenes), viruses (e.g. enteroviruses, herpesviruses an arboviruses), fungi (e.g. *Cyptococcus spp.),* and parasites (e.g. some species of amoebae).

**Who is at risk?**

Meningitis can affect anyone anywhere, and at any age. The pathogens that cause it can vary, based on a person's age and immune system, and level of exposure to risk, which can be influenced by their living conditions and geographical location.

Newborn babies are most at risk from Group B streptococcus, whereas children and adolescents are at most risk of meningococcus, pneumococcus and *Haemophilus influenzae*. Pneumococcus and meningococcus also account for most cases of bacterial meningitis among adults.

Immunocompromised and/or people living with HIV are at increased risk of different types of meningitis.

Globally, the highest burden of disease is seen in a region of sub-Saharan Africa, known as the African meningitis belt, which stretches from Senegal to Ethiopia, and is at high risk of recurrent epidemics of meningococcal meningitis.

Meningococcal meningitis outbreaks occur more frequently under special risk conditions, such as crowded settings where people are in close proximity, mining areas, mass gatherings, such as religious or sporting events, settings with refugees or displaced persons, closed institutions, military camps and areas with high migration, such as high-traffic markets and border areas.

**Transmission**

The route of transmission varies by organism. Most bacteria that cause meningitis, including meningococcus, pneumococcus and *Haemophilus influenzae*, are carried in the human nose and throat. They are spread from person to person by respiratory droplets or throat secretions. Group B streptococcus is often carried in the human gut or vagina and can spread from mother to child around the time of birth.

Carriage of these organisms is usually harmless and contributes to building up immunity against infection, but the bacteria occasionally invade the body, causing meningitis, sepsis and other forms of invasive disease.

**Signs and symptoms**

The symptoms of meningitis can differ based on the cause, how quickly the disease progresses, how long it lasts, brain involvement, and other serious complications like sepsis.

Common symptoms of meningitis are fever, neck stiffness, confusion or altered mental status, headache, sensitivity to light, nausea and vomiting. Less frequent symptoms include seizures, coma and neurological deficits, such as weakness of the limbs.

Infants often have different symptoms compared to adults:

- unusual behaviour, such as the child being less active and difficult to wake
- irritability
- weak, continuous cry
- poor feeding
- bulging of the soft spot in their head.

Some bacterial pathogens may also account for other symptoms as a result of bloodstream infection, which can quickly lead to sepsis, including cold hands and feet, fast breathing and low blood pressure. A characteristic, non-blanching skin rash may appear with meningococcal sepsis.

**Complications and sequelae**

One in 5 people surviving an episode of bacterial meningitis may have long lasting after-effects. These after-effects include hearing loss, seizures, limb weakness, difficulties with vision, speech, language, memory and communication, as well as scarring and limb amputations after sepsis.

**Prevention**

Vaccines offer the best protection against common types of bacterial meningitis.

Vaccines can prevent meningitis caused by:

- meningococcus
- pneumococcus
- *Haemophilus influenzae* type b (Hib).

Maternal Group B streptococcus vaccines to prevent invasive GBS disease in infants are in the final stages of clinical development.

Bacterial and viral meningitis can spread from person to person. If you live with someone who has either type of meningitis, you should:

- talk to your doctor or nurse about taking antibiotics (in case of bacterial meningitis)
- wash hands frequently, especially before eating
- avoid close contact and sharing cups, utensils or toothbrushes.

**1. Vaccination**

Licensed vaccines against meningococcal, pneumococcal and *Haemophilus influenzae* disease have been available for many years. These bacteria have several different strains (known as serotypes or serogroups) and vaccines are designed to protect against the most harmful strains. No universal vaccine exists.

Hib vaccine is used in most national childhood immunization programmes globally. WHO also recommends universal use pneumococcal conjugate vaccines (PCV). Meningococcal vaccines include multivalent polysaccharide conjugate vaccines (MMCV), which include 4 to 5 meningococcal serogroups (A,C,W,Y,X); protein-based vaccines, which include meningococcal serogroup B, and combination vaccines combining the latter with -4valent MMCV. Polysaccharide vaccines are still marketed internationally but are gradually being replaced by MMCV.

In the African meningitis belt, meningococcus serogroup A accounted for 80–85% of meningitis epidemics before the large-scale deployment of a meningococcal A conjugate vaccine starting in 2010. In 2023, the first pentavalent MMCV protecting against serogroups A, C, W, Y and X (Men5CV) was prequalified by WHO and recommended for use in

countries of the African meningitis belt. Roll-out of Men5CV has the potential to eliminate meningitis epidemics and make the meningitis belt history.

**2. Antibiotics for prevention (chemoprophylaxis)**

Post-exposure prophylaxis with antibiotics is given to close contacts of individuals with meningococcal disease to eradicate asymptomatic meningococcal carriage in the nose and decrease the risk of transmission.

Identifying mothers whose babies are at risk of getting Group B streptococcal (GBS) disease is recommended in many countries. Mothers at risk of transmitted GBS to their babies are offered intravenous penicillin during labour to prevent their babies developing GBS infection.

**Diagnosis**

To diagnose meningitis, a lumbar puncture is needed to examine the cerebrospinal fluid (CSF). This should be done before starting antibiotics; however, if bacterial meningitis is suspected based on the signs and symptoms, a lumbar puncture should never delay antibiotic treatment.

Laboratories will then perform specific tests with CSF or blood to identify the pathogen causing the infection. The tests will also help identify the treatments needed, and specifically for bacterial infections the susceptibility to types of antibiotics, as well as identify the strain(s) of the pathogen responsible and inform public health responses.

**Treatment**

Meningitis is a medical emergency and requires urgent medical attention in an appropriate health-care facility.

Antibiotic treatment should be started as soon as possible when bacterial meningitis is suspected. The first dose of antibiotic treatment should not be delayed until the results of the lumbar puncture are available. The choice of antibiotic treatment should consider the age of the patient, presence of immunosuppression, and local prevalence of antimicrobial resistance patterns. In non-epidemic settings, intravenous corticosteroids (e.g., dexamethasone) are initiated with the first dose of antibiotics to reduce the inflammatory response and the risk of neurological sequelae and death,

Those who have lived through meningitis can have complications such as deafness, learning impairment or behavioural problem and require long-term treatment and care. The ongoing psychosocial impacts of disability from meningitis can have medical, educational, social and human rights-based implications. Access to both services and support for these conditions is often insufficient, especially in low- and middle-income countries.

Individuals and families with members disabled by meningitis should be encouraged to seek services and guidance from local and national organizations of disabled people and other disability focused organizations, which can provide vital advice about legal rights, economic opportunities and social engagement to ensure people disabled by meningitis are able to live full and rewarding lives.

WHO has also developed an Intersectoral global action plan on epilepsy and other neurological disorders to address the many challenges and gaps in providing care and services for people with epilepsy and other neurological disorders that exist worldwide, including those suffering from meningitis sequelae.

**Surveillance**

Surveillance, from case detection to investigation and laboratory confirmation, is essential to the control of meningitis. Main objectives include:

- detect and confirm outbreaks;
- monitor the incidence trends, including the distribution and evolution of serogroups and serotypes;
- estimate the disease burden;
- monitor the antibiotic resistance profile;
- monitor the circulation, distribution, and evolution of specific strains (clones); and
- estimate the impact of meningitis control strategies, particularly preventive vaccination programmes.

**WHO response**

In 2020, the 73rd World Health Assembly approved resolution (WHA73.9), in which all Member States committed to implementing the Defeating meningitis by 2030 global road map.

The roadmap sets a comprehensive vision "Towards a world free of meningitis" and has 3 visionary goals:

- elimination of bacterial meningitis epidemics;
- reduction of cases of vaccine-preventable bacterial meningitis by 50% and deaths by 70%; and
- reduction of disability and improvement of quality of life after meningitis due to any cause.

## 5. Post COVID-19 condition (long COVID)

**KEY FACTS**

- Most patients with COVID-19 recover fully, but some develop post COVID-19 condition with medium- to long-term effects on one or more body systems.
- Approximately 6 in every 100 people who have COVID-19 develop post COVID-19 condition.
- While data are limited, the chance of developing post COVID-19 condition appears to be lower now than earlier in the pandemic. However, the virus is still circulating widely, and every new infection is associated with a risk.

- Fatigue, breathlessness, muscle or joint pain, and impaired sleep are common symptoms of post-COVID-19 condition.
- WHO is working to develop clinical practice guidelines for management of post COVID-19 condition.
- Health-care providers can guide patients on self-management of symptoms and offer medication for symptom relief or referral for rehabilitation services as needed.

**Overview**

COVID-19 can lead to serious long-term effects, known as post COVID-19 condition (PCC). It is also commonly referred to as long COVID. Post COVID-19 condition is characterized by a range of symptoms which usually start within 3 months of the initial COVID-19 illness and last at least 2 months. PCC can affect a person's ability to perform daily activities such as work or household chores and restrict social participation.

**Scope of the problem**

Millions of people have been affected with post COVID-19 condition since the beginning of the pandemic *(1)*. Global estimates indicate that 6 in 100 people with COVID-19 develop post COVID-19 condition. Estimates largely come from people who suffered COVID-19 early in the pandemic (in the first two years), and there is a very large variation in estimates *(2)*.

More recent research shows the chances of developing post COVID-19 condition have reduced, but these data are limited and mostly from high-income countries *(3)*. However, the SARS-CoV-2 virus, the virus that causes COVID-19 is widely circulating and post COVID-19 condition remains a substantial threat and ongoing challenge to global public health.

**Risk factors**

Anyone who was infected with SARS-CoV-2 can develop post COVID-19 condition. Some people have higher risk. These include women, older adults, smokers, those who are overweight or obese or have pre-existing chronic health problems. Repeated infections and severe COVID-19 needing hospitalization or ICU admission also increase the risk *(4)*. We see higher numbers of post COVID-19 condition sufferers among people with disabilities, and where health disparity and access to health care is a problem *(5)*.

Research is ongoing to better understand what causes post COVID-19 condition. Almost any organ can be affected, including the heart and blood vessels, lungs, nervous system, gut and endocrine (hormone) system. In those with post COVID-19 condition, researchers have found evidence of persistence of SARS-CoV2 virus in the body, of altered immune responses and autoimmunity, and of formation of microscopic blood clots (micro-thrombosis), among other problems *(6)*.

**Symptoms**

Over 200 different symptoms have been reported by people with post COVID-19 condition. Common symptoms include:

- fatigue
- aches and pains in muscles or joints
- feeling breathless
- headaches
- difficulty in thinking or concentrating
- alterations in taste.

Impaired sleep, depression and anxiety also occur *(5)*. These symptoms might persist from their initial illness or develop after their recovery. Symptoms can be mild to severely debilitating, and affect someone's capacity to work, perform their daily activities or do exercise.

With increasing understanding of post COVID-19 condition, some clinical patterns have become clearer. There are symptoms which tend to occur together, for example dizziness, palpitations, light-headedness on standing, and exercise intolerance (related to postural orthostatic tachycardia syndrome), symptoms of post exertional malaise, or myalgic encephalomyelitis/ chronic fatigue syndrome (ME/CFS) *(7)*.

Other medical conditions can occur more often than usual after having COVID-19. These include kidney impairment, heart disease, stroke, diabetes and mental health disorders, among other conditions *(8)*.

**Impact**

Post COVID-19 condition can affect the ability to work and may lead to loss of productivity, and a reduction in income and quality of life. Ongoing medical needs of people with the condition may stretch existing health systems.

**Recovery**

Symptoms of post COVID-19 condition generally improve over time, typically 4–9 months. Approximately 15 in 100 people still have symptoms at 12 months as per global estimates from 2022 *(2)*.

**Treatments**

The individual needs of patients with post COVID-19 condition vary. At the present time, there remains limited research on treatments and a lack of large studies to understand the most effective

treatments. However, doctors and their patients may make individualized treatment decisions based on knowledge from similar medical conditions. Health-care providers may offer medications for symptomatic relief as needed. Additionally, newly diagnosed medical problems occurring after COVID-19 frequently have well-established treatments, for example kidney disease or stroke. Many symptoms and functional impairment can be managed effectively by rehabilitation, and with careful communication between primary care practitioners and medical specialists.

**Self-care**

Education about the importance of quality rest and sleep and skills training on energy conservation techniques can help patients manage their symptoms better. Health-care providers can discuss with patients about self-management strategies to respond promptly to a flare-up or relapse, such as identifying possible triggers, temporarily reducing activity levels, monitoring symptoms over time, and not returning to usual activity levels until the flare-up has resolved. Use of assistive devices and environmental modifications at work and home may be needed in some instances.

**Prevention**

People can be reinfected with SARS-CoV-2 multiple times. Each time, they have a risk of developing post COVID-19 condition. Therefore, risk reduction with preventive measures such as the use of masks, personal hygiene and ventilation in high-risk situations continues to be important. Receiving two doses of vaccination appears to reduce the likelihood chance of developing post COVID-19 condition *(9)*.

**WHO response**

WHO first started work on post COVID-19 condition in the first wave of the global COVID-19 pandemic in 2020 when reports began to emerge that some patients had persistent symptoms weeks or months following SARS-CoV-2 infection. To better understand this phenomenon, WHO met with patient advocates, researchers, health-care providers and public health professionals and by September 2020, established emergency international classification of disease (ICD) codes for post COVID-19 condition.

Since then, WHO has developed a clinical case definition of post COVID-19 condition to recognize the condition and its impact on people's lives. This definition was developed by patients, researchers and clinical experts, representing all WHO regions, with the understanding that the definition may change as new evidence emerges and our understanding of the consequences of COVID19- evolves.

A separate clinical case definition for post COVID-19 condition in children and adolescents is also available.

WHO has been organizing webinars on post COVID-19 condition since February 2021 to expand understanding of the condition and its impact on patients' lives, and foster research and collaboration. A global webinar series on the medical management of post COVID19- condition is organized each month and has been running since 2023.

A WHO Guideline Development Group consisting of global experts, frontline providers and affected individuals is presently at work on guidelines on diagnosis, treatment and rehabilitation in post COVID19- condition.

We advocate for governments and funders to support research on post COVID-19 condition in the interest of improved understanding of this condition around the world, not just in high-income countries, and design optimal clinical care for patients. National authorities are encouraged to plan and budget for multidisciplinary post COVID-19 condition programmes and to ensure equitable access to relevant therapies.

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