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REVIEW

Nanomedicine approaches for treatment of hematologic and oncologic malignancies

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Abstract

Cancer is a leading cause of death worldwide. Nowadays, the therapies are inadequate and spur demand for improved technologies. Rapid growth in nanotechnology and novel nanomedicine products represents an opportunity to achieve sophisticated targeting strategies and multi-functionality. Nanomedicine is increasingly used to develop new cancer diagnosis and treatment methods since this technology can modulate the biodistribution and the target site accumulation of chemotherapeutic drugs, thereby reducing their toxicity. Cancer nanotechnology and cancer immunotherapy are two parallel themes that have emerged over the last few decades while searching for a cure for cancer. Immunotherapy is revolutionizing cancer treatment, as it can achieve unprecedented responses in advanced-stage patients, including complete cures and long-term survival. A deeper understanding of the human immune system allows the establishment of combination regimens in which immunotherapy is combined with other treatment modalities (as in the case of the nanodrug Ferumoxytol). Furthermore, the combination of gene therapy approaches with nanotechnology that aims to silence or express cancer-relevant genes *via* one-time treatment is gradually progressing from bench to bedside. The most common example includes lipid-based nanoparticles that target VEGF-A and KRAS pathways. This review focuses on nanoparticle-based platforms utilized in recent advances aiming to increase the efficacy of currently available cancer therapies. The insights provided and the evidence obtained in this paper indicate a bright future ahead for immuno-oncology applications of engineering nanomedicines.

Key Words: Nanomedicine; Cancer; Immunotherapy; Gene; Cell therapy

Core Tip: Despite many years of fundamental and clinical examination and preliminaries of promising new treatments, cancer stays a significant reason for dreariness and mortality. Ongoing investigations propose that nanomedicine gives benefits over conventional treatments for cancer therapy. Immunotherapeutic strategies, such as cancer vaccines, immunomodulatory agents, immune checkpoint inhibitors, natural killer cells, peptides, nucleic acids, and chimeric antigen receptor T-cells, have augmented the development of this treatment either by stimulating cells or blocking the so-called immune checkpoint pathways. The efficacy of nanomedicine treatments and the examination of the advancement in the synergistic plan of immune-targeting combination therapies reviewed in this manuscript have been validated in clinical trials. The field of nanomedicine, therefore, generates new approaches regarding oncologic malignancies.

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INTRODUCTION

Cancer refers to a diverse group of more than 100 different diseases that exhibit a highly complex and multifactorial profile and together serve as one of the leading causes of death worldwide, accounting for nearly 10 million deaths in 2020 alone[1]. The global prevalence of cancer was estimated to rise from 17 million cases annually in 2018 to 27.5 million in 2040[2]. Although the etiology of each type is hugely varying and the clinical manifestations quite heterogeneous, aberrated cellular and tissue regulation is among the trademarks of cancer[3]. Fundamentally, each type results in the accumulation of genetic and epigenetic alterations that dysregulate the cell cycle and promote abnormal cell growth[4].

Collectively, these alterations impair cellular control mechanisms and the responsible regulatory signaling pathways and drive the transformation of normal cells into malignant cells. The malignant cells acquire new biological abilities, referred to as the hallmarks of cancer[5], a set of distinct features including sustained proliferation, evasion of growth suppression and cell death, altered response to metabolic and stress cues, vascularization, invasion and metastasis, and immune modulation. Various genes have been implicated in carcinogenesis, from activated oncogenes and anti-apoptotic genes to inactivated tumor suppressor genes[6,7]. Large-scale genomic analysis and functional studies have facilitated the identification of distinct mutations in different tumor types, allowing the development of valuable diagnostic biomarkers[8] and the stratification of patients towards more personalized therapeutic approaches[9].

The field of oncology focuses on the prevention, diagnosis, and treatment of cancer while implementing various strategies and tools for clinical application. Conventional cancer therapeutics, including chemotherapy, radiation therapy, and surgery, are widely accepted and used; however, they suffer from various drawbacks due to the lack of tumor-specific targeting, dosing, pharmacokinetic limitations, and severe complications[10]. Concurrently, a tremendous body of research work has been generated over the last decades to advance on the one hand, the understanding of the complicated events governing tumorigenesis[11] and, on the other hand, to develop early diagnostic and efficient therapeutic approaches[12]. Towards that goal, a promising branch of biomedicine, nanomedicine, aims to boost these current cancer management strategies. Nanomedicine can be defined as the use of nanomaterials (materials with at least one dimension ranging from 1-100 nm) for the prevention, diagnosis, and ultimately, treatment of diseases[13].

Nanomedicine, the application of nanotechnology in medical practice, aims to bridge the gap between different scientific principles such as physics, chemistry, pharmaceutics, and biology to harness nanotechnology's knowledge and tools with the goal of serving medicine. The unique phenomena that govern the nanoscale enable novel medical applications and are responsible for the exceptional properties that make nanomaterials excellent candidates for therapeutic applications[14]. Despite their minuscule size, nanoparticles hold great potential as drug delivery systems for cancer treatment, and tremendous research has taken place in the last decades to bring this technology from bench to bedside [15].

Nanoparticles as drug carriers have proven to be an effective tool in the fight against cancer[16]. The improved selectivity afforded by these nanocarriers resulted in a significant increase in the efficacy of the carried medicine, while side effects in the host were minimized. It is also feasible to include

targeting moieties specific for cell organelles, which boosts the efficacy of the transported medicines even more[17]. Nanoscale platforms come in various sizes, geometries, materials, and targeting moieties, allowing them to target organs, tissues, and individual cells[18]. Because of their distinct benefits, nanomedicines have emerged as a viable alternative to viral vectors, including low toxicity and immunogenicity, sustained and controlled release features, scale-up capacity, and low-cost manufacturing[17].

This review thus focuses on nanoparticle-based platforms utilized in recent advances aimed to increase the efficacy of currently available cancer therapies.

TYPES OF CANCER NANOMEDICINES

Immunomodulatory agents

Recently, some of the alternate approaches to treat cancer are based on immunomodulation which employs the host's own natural defense mechanisms to recognize and selectively eliminate the cancer cells by inducing the immune system[19]. Nanomaterial-mediated immunomodulation can be achieved either directly or indirectly[20]. To the first group belong nanomaterials that act as vaccine adjuvants, as several systems have been reported to improve antigenicity of conjugated weak antigens, while engineered nanosystems have also shown inherent antigenic properties[21]. Recent studies have highlighted the inherent tendency of liposomes to interact extensively with the immune system leading to several immunomodulatory effects, concerning tumor growth[22]. More specifically, circulating proteins are rapidly integrated to the surface of liposomes, forming a protein corona which can function as the interface for biological interactions and contributes to the formation of immune complexes and immunogenic epitope generation from self-antigens, ultimately resulting in the activation or suppression of immune responses[22]. Moreover, increasing evidence is emerging that indicates the functional ability of nanoparticles to polarize macrophages[23]. On the other hand, the multicomponent cargo capacity of delivering immunomodulatory agents in a targeted manner enables their function as delivery platforms that bolster the immune response.

Nanomaterials used in the combating of the immune evasion strategies of cancer operate in three different approaches that include the immunogenic targeting of cancer cells, the reshaping of the tumor's immune microenvironment, and the stimulation of the peripheral immune network[24].

When targeting cancer cells, nanomedicines typically aim to induce immunogenic cell death (ICD), thereby triggering an immunogenic cascade that leads to an antigen-specific immune response against a broad spectrum of solid tumors. It is now established that ablative cancer treatments, such as radiotherapy, photodynamic therapy, hyperthermia, and photothermal therapy, as well as certain chemotherapeutics can cause tumor cell death[11].

In the context of tumor immune microenvironment (TIME), nanomaterials can be used to modulate the immunosuppressive tumor microenvironment by targeting tumor-associated macrophages (TAMs), regulatory T cells (Treg cells), regulatory B cells, myeloid-derived suppressor cells (MDSCs), as well as cancer-associated fibroblasts. Several nanoparticle-based strategies that target TAMs for suppressing tumor progression include TAM depletion, inhibiting monocyte recruitment, and TAM reprogramming [25]. Recent studies showed that the utilization of dendrimer nanoparticles carrying the chemotherapeutic methotrexate that specifically recognize the folate receptor-2, which is overexpressed in TAMs, increases therapeutic efficacy by depleting TAMs[25]. Considering monocyte recruitment, it is reported that silver nanoparticles have an adjuvant effect inducing recruitment and activation of local macrophages[26]. As for the reprogramming of macrophages, there have been attempts for creating an albumin-derived nanoplatform that delivers both the disulfiram/copper complex and macrophage modulator regorafenib for reprogramming macrophage[27]. In the context of down-regulating Treg cells, a common strategy is the use of checkpoint blockade antibodies (anticytotoxic T lymphocyte-associated protein 4)[25]. A modulating strategy for abnormal MDSC differentiation has been introduced, using lipid-coated biodegradable hollow mesoporous silica nanoparticles[28] in order to induce differentiation of MDSCs to mature DCs, macrophages, and granulocytes.

Nanomedicines can furthermore be applied in cancer vaccination to target the peripheral immune system[29]. This application's grounds are based on the notions that intradermally or subcutaneously injected nanoparticles drain to LNs and that antigens bound to a nanoparticle are more efficiently processed by APCs. Instead of triggering APCs to present antigens to naive T cells, nanomedicines have also been designed to replace APCs by directly generating cytotoxic T cells[29].

During the last decade, nanoparticle-based immunotherapy formulations have passed from the preclinical stage in the clinical trials and several new treatments have been approved. Ferumoxytol is a nanoparticle formulation that contains iron oxide cores that are coated with carboxymethyl dextran. It enhances the production of reactive oxygen species by macrophages *via* the Fenton reaction, as M1 macrophages release hydrogen peroxides[30]. As an outcome, cancer cell cytotoxicity is enhanced while continued M1 polarization triggered by apoptotic cancer cells creates an autocrine feedback loop that maintains the production of tumor necrosis factor. Because ferumoxytol is FDA-approved, the drug is accessible for cancer patients through 'off-label' use[30]. The only cancer vaccines currently in routine

clinical use are the Sipuleucel-T and the Talimogene laherparepvec (T-VEC). The FDA-approved nanomedicine for the treatment of prostate cancer is sipuleucel-T which is a personalized vaccine encompassing patients' *ex vivo* processed dendritic cells that express a key tumor antigen, prostatic acid phosphatase (PAP)[31,32]. T-VEC is an engineered oncolytic herpes simplex virus type 1 in which the neurovirulence factor ICP34.5 is replaced by the coding sequence for GM-CSF and acts as a single agent in patients with skin and soft tissue metastases[32]. GM-CSF functions to recruit antigen presenting cells to the tumor microenvironment and promote cytotoxic T-cell responses to tumor associated antigens.

Immune checkpoint inhibitors

The last decade cancer immunotherapies have changed the perspective of cancer treatment (Table 1). The basic immunotherapy options approved are immune checkpoint inhibitors (ICIs).

ICIs have stirred up the field of tumour therapy and are now considered first-line therapies for various solid and liquid tumours. The approval of anticytotoxic T lymphocyte-associated protein 4 for advanced stage melanoma in 2011, opened up a new field of exploration that led to the 2018 Nobel Prize in Medicine to James P. Allison and Tasuku Honjo for inhibiting negative immune regulation in cancer [33].

Cancer immunotherapies are defined as therapies that directly or indirectly target any component of the immune system that is involved in the anti-cancer immune response, including the stimulation, enhancement, suppression, or desensitization of the immune system. These therapies are composed of monoclonal antibodies targeting the cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and PD-1 receptors and the PD-1 ligand PD-L1, which are involved in the regulation of T cells. As shown in Table 1, there is a plethora of ICIs approved for the treatment of various cancer.

As a general concept, T cell activation needs two signals, first theantigen recognition by the T cell receptor (TCR), and then the antigen presentation by major histocompatibility complex class II molecules on the surface of antigen-presenting cell that leads to signal modulation by CD80 or CD86 binding to the CD28 receptor[34].

CTLA-4 is found on the T cell surface competing with the CD28 receptor to bind CD80 or CD86, thereby blocking T cell activation. Furthermore, CTLA-4 inhibitors block CTLA-4-CD80 or CTLA-4-CD86 binding to facilitate T cell activation (Figure 1A). In Figure 1B, we see PD-1 as a surface receptor that is expressed by T cells and promotes apoptosis of antigen-specific T cells and reduces apoptosis of regulatory T cells through its interaction with its ligand, PD-L1, which is expressed by tumour cells and myeloid cells[35,36]. This interaction is useful in preventing autoimmunity in physiological conditions, but cancer cells exploit this process to escape from immune system activity, upregulating PD-L1 expression[37,38]. PD-1 and PD-L1 inhibitors disrupt the PD-1-PD-L1 interaction, facilitating T cell activation and survival (dashed lines).

The use of ICIs for cancer therapy is increasing; however, only a minority of patients treated with ICIs achieve a durable response. A portion of patients that receive ICIs do not respond to treatment, while others respond initially but ultimately acquire resistance. Primary and acquired resistance are the effect of constantly changing interactions among cancer cells and the immune system. Even in patients with melanoma, which has one of the highest rates of response to ICI, 60%-70% of patients do not experience an objective response to anti-PD-1 therapy[39,40]. Moreover, 20%-30% of patients demonstrate eventual tumour relapse and progression. A key challenge that has emerged with the progressive implementation of ICIs in clinical practice is their uncontrolled collateral effects on the immune system that can lead to so-called immune-related adverse events (irAEs).

ICIs have a different spectrum of toxicities[41] from standard chemotherapy or other biological agents, and most toxicities result from excessive immunity against normal organs. All the primary and secondary (acquired) resistance are a result of complex and constantly evolving interactions between cancer cells and the immune system. The most frequently noted irAEs involve inflammation of gastrointestinal, dermatologic, endocrine, or pulmonary organs. Several clinical trials for ICIs including adjuvant and neo-adjuvant therapies are still in progress.

The role of nanomedicine in ICIs is to ensure an increased therapeutic outcome by using specific nanocarriers. Several formulations are currently investigated in both pre-clinical and clinical studies [42]. Starting from the preclinical studies, at least 12 different nanocarriers are being investigated. These include gold nanostars that are being tested for the PD-L1 blockage, PLGA combined with anti-CTLA4, and incorporation of anti-PD-1 and anti-TIM-3 with liposomes[43-46]. All these preclinical studies are held in mouse models for bladder, breast (4T1 cells), and colon cancer.

An important number of clinical trials are also being performed. These mainly include Nanoparticle Albumin Bound (Nab) formulations that combine ICIs with standard chemotherapeutics (as in the case of paclitaxel and carboplatin in Pembrolizumab, Atezolizumab, and Nivolumab formulations). Non-Nab strategies in nanoparticle-based immunotherapy include the radiosensitizer molecule NBTXR3[42].

Most of these studies will require a considerable amount of time to be completed and for the efficacy of these nanoformulation to be investigated. The poor lymphatic drainage of tumours (part of a phenomenon known as the EPR effect) could theoretically offer an advantage of nanoformulations over conventional ones although due to its complicated nature of EPR, this needs to be validated in these studies[47,48]. At the same time, further considerations are constantly being applied for future applications as the concept of smart nanoplatforms that will be triggered only upon an external stimulus[49-

Table 1 Approved immune checkpoint inhibitors according to cancer type

Anti-CTLA-4 antibodies
Ipilimumab; Colorectal cancer; Melanoma; Renal cell carcinoma
Anti-PD-1 antibodies
Nivolumab; Bladder cancer; Colorectal cancer; Head and neck cancer; Hepatocellular carcinoma; Hodgkin lymphoma; Melanoma; Non-small-cell lung cancer; Renal cell carcinoma; Cemiplimab; Cutaneous squamous cell carcinoma; Pembrolizumab; Bladder cancer; Cervical cancer; Gastro-oesophageal junction cancers; Head and neck cancer; Hepatocellular carcinoma; Hodgkin lymphoma; Merkel cell carcinoma; Metastatic solid tumours classified as microsatellite instability high or deficient mismatch repair; Non-small-cell lung cancer; Primary mediastinal large B cell lymphoma; Stomach cancer
Anti-PD-L1 antibodies
Atezolizumab; Bladder cancer; Breast cancer; Non-small-cell lung cancer; Avelumab; Bladder cancer; Merkel cell carcinoma; Durvalumab; Bladder cancer; Non-small-cell lung cancer

Anti-CTLA-4: Anticytotoxic T lymphocyte-associated protein 4; PD-1: Programmed cell death protein - 1.

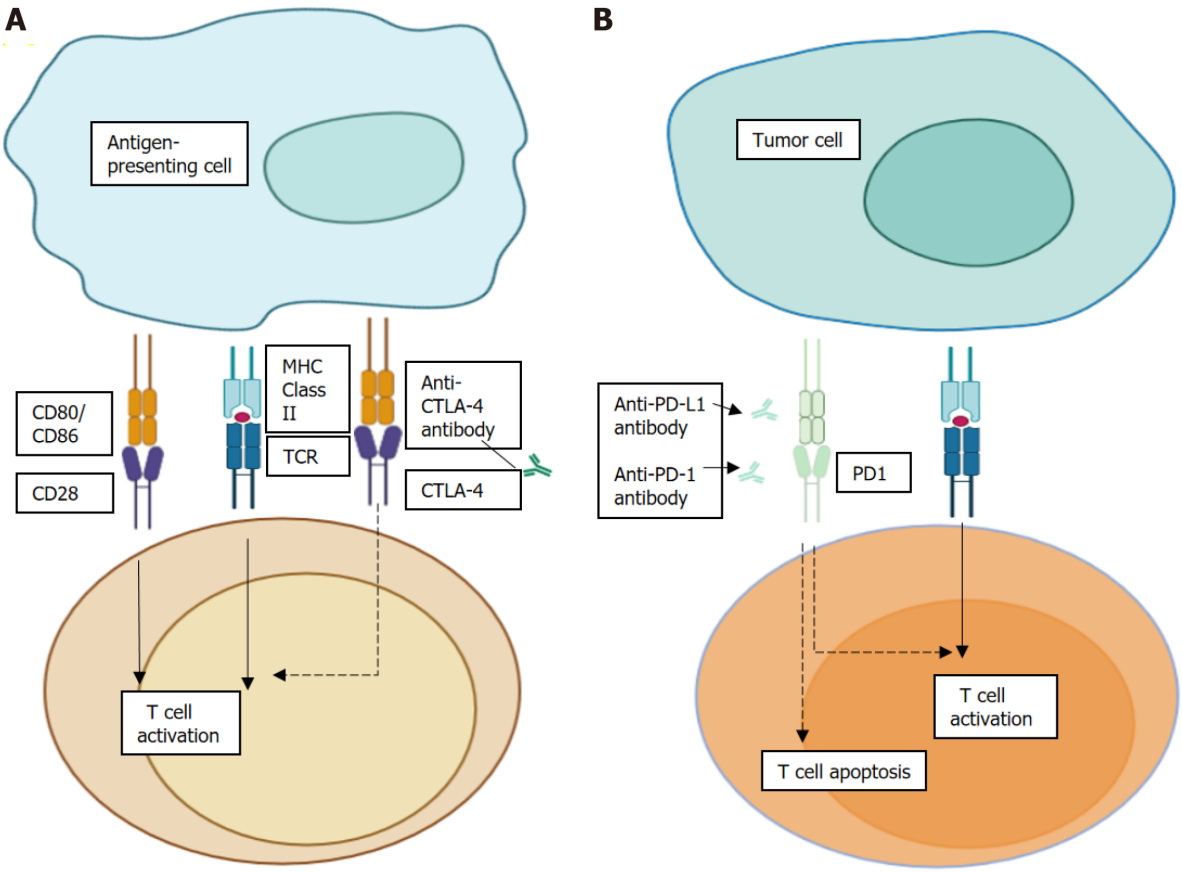

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Figure 1 Mechanism of action of immune checkpoints and immune checkpoint inhibitors. A: Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) inhibitors block CTLA-4-CD80 or CTLA-4-CD86 binding to facilitate T cell activation; B: We see PD-1 as a surface receptor that is expressed by T cells and promotes apoptosis of antigen-specific T cells and reduces apoptosis of regulatory T cells through its interaction with its ligand, PD-L1, which is expressed by tumour cells and myeloid cells. CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; MHC: Major histocompatibility complex; PD1: Programmed cell death protein 1.

51].

Triggers of natural killer cells

Cancer immunotherapy is considered to eliminate primary as well as metastatic tumors and it is shown to develop immunological memory. It is important to say that nanomedicine can deliver a vast number of immunological agents to the targeted site (*i.e.*, tumor)[52]. Nanomedicines have been explored thoroughly for tumor-targeted drug delivery and reducing the side effects of chemotherapeutic drugs. Tumor targeting is mainly mediated by passive targeting and/or active targeting and has been evaluated based on the average targeting efficiency and clinical impact (Table 2). There are three targeting strategies to boost cancer immunotherapy, including targeting and killing cancer cells to

Table 2 Clinical trials on natural killer cells in hematological and solid tumors

Condition	Interventions	Phase	Ref.	Status
Solid tumor	ROBO1 CAR-NK cells	I/II	NCT03940820	Recruiting
Ewing sarcoma; Neuroblastoma; Rhabdomyosarcoma; Osteosarcoma; CNS tumors	Allogeneic HCT; Donor NK cell infusion	II	NCT02100891	Active, not recruiting
Brain and CNS tumors; leukemia; lymphoma; chronic myeloproliferative disorders; lymphoproliferative disorder multiple myeloma and plasma cell neoplasm; myelodysplastic syndrome; myelodysplastic/ myeloproliferative neoplasm; unspecified adult solid tumor, protocol specific	Donor NK cell infusion	I/II	NCT00823524	Completed
Malignant solid tumors	NK Immunotherapy	II	NCT02853903	Completed
Malignant solid tumors	NK Immunotherapy	I/II	NCT02857920	Completed
Multiple myeloma	CIML NK cells plus KP1237 and low dose IL-2	I/II	NCT04634435	Recruiting
Hematological malignancy;	NK cell infusion	I	NCT01853358	Completed
leukemia; lymphoma; myeloma; Hodgkin's disease	NK-92 cells	I	NCT00990717	Completed
Acute lymphoblastic leukemia; chronic lymphoblastic leukemia; B-cell lymphoma	Fludarabine + Cyclophosphamide + CAR-NK-CD19 Cells	I	NCT04796688	Recruiting
Leukemia; lymphoma	NK cell infusion	I	NCT01287104	Completed

NK: Natural killer.

induce specific forms of ICD, TIME, and targeting the peripheral immune system[53].

Natural killer (NK) cells, part of the innate immune system, have been identified as the next-generation therapy for cancer. These cells are lymphocytes with antitumor and antiviral abilities that have several applications. NK cells have memory-like and memory responses after cytokine preactivation, viral infections, and hapten exposure, in addition to being classified as innate lymphoid cells [54]. They have various mechanisms for directly killing cancer cells and enhancing the immune system's ability to fight cancer. Over the last 40 years, NK cell immunotherapy has demonstrated encouraging effects in both preclinical and clinical studies. These cells have been used for years and have been approved by the FDA. The NK-92 cell line (CD56+/CD3-), isolated from a patient with lymphoma, has expected high cytotoxic movement and can be extended under acceptable assembling practice conditions in recombinant interleukin-2[55].

Many nanoparticles have been discovered to be immunotherapy carriers, delivering antitumor immunotherapeutics specifically to tumor cells. These nanoparticles could provide stability, increase solubility, and cause less toxicity to healthy cells. Nanoparticles have the potential to deliver immunotherapeutics directly to cancer sites, which can be explained by their increased duration in the bloodstream without altering the body's physiochemical properties. The lymphoid node secures the nanoparticles prior to their drug conveyance priority and the elimination of toxic waste products. When nanoparticle immunotherapy is used passively to target cancer, there is a significant reduction in cellular cytotoxicity and a favorable outcome. Thus, to achieve an effective outcome, the delivery system must be modified so that the immunotherapeutic carrier enters the intracellular space before accomplishing the immunotherapy. There are several types of nanoparticles, which are classified based on their size, morphology, and physical and chemical properties[56]. Nanotechnology, specifically nanoparticles as drug delivery systems (DDSs), eases targeted medicines and theragnostics. Most nanomedicines include a targeting element, but some do not, yet[57].

Magnetic nanoparticles, Fe_3O_4, were modified with meso-2,3-dimercaptosuccinic acid, as the affinity of the electron-rich carboxyl group was higher and the orbital in the Fe atom was empty. After obtaining CD56 antibody-modified Fe_3O_4 nanoparticles aided by a shorter co-culture period, NK-92 cell recruitment and infiltration into solid tumors were improved in the presence of a magnetic field. Biohybrid treatment with NK-CD56 nanoparticles effectively suppressed tumor growth and significantly prolonged the survival of cancer-bearing mice. Finally, by synergizing immune cells with a directional magnetic field that promotes infiltration into solid tumor tissue under magnetic resonance imaging control, antitumor efficiency is significantly improved. Magnetic nanoparticles and NK cells can be utilized for various biomedical applications, as they have proved to possess flexible characteristics to operate in biomedicine[58].

Gold nanoparticles (AuNPs) were coated with PEG and D-(β)-glucosamine, as glucose coating increases the cellular uptake of the nanoparticles. The K562 human erythroleukemia cell line (positive target) and the 888 human melanoma cell line (negative control) were co-cultured with AuNP-labeled NK-92 cells. The results indicate that AuNP-labeled NK-92 cells can specifically identify target cells and

keep their cytokine secretion and antitumor function. In addition, gold nanoparticles do not undermine the therapeutic effect of NK-92 cells *in vivo*. AuNPs could assist NK cells to achieve their aim, the regression of the tumor; therefore, this combinatorial therapy for cancer will reduce or even end the dosage of radiation[59].

CHIMERIC ANTIGEN RECEPTOR T-CELL BASED TREATMENTS

Worldwide, approximately 97% of active clinical trials are chimeric antigen receptor (CAR) T-cell-based therapies. Nanoparticles can engineer NK cells to produce CAR-NK therapy by targeting several ligands, such as antibodies to nanoparticles, and accomplish successful targeted delivery[60]. Axicabtagene ciloleucel (refractory diffuse large B-cell lymphoma) and tisagenlecleucel (B-cell precursor acute lymphoblastic leukemia) were two CAR T-cell therapies that cure blood cancer approved by the FDA [61]. Functional antitumor immune response has been shown by adoptive cell transfer studies, such as CAR T-cell therapy [62].

PEPTIDES

Peptides are a powerful tool in cancer diagnosis and treatment with many advantages and numerous ways to alter their function and use them in oncology. They present with excellent biocompatibility (degradation products are amino acids, which are a natural source of cells). They can be formulated and introduced with all kinds of modifications. By using the process of self-assembly, we can improve the stability of a peptide sequence and create the conditions for better targeting of the diseased organ. Their big advantage depends on their small size and better tissue/cell penetration[63,64]. To minimize the nonselective side effects of chemotherapy, a specific peptide sequence or motif can be used. Nanoparticles based on peptides, can be used to target cancer cells, to minimize systemic drug exposure and increase efficiency of the drug that is to be delivered[63].

Some examples of therapeutic peptides in clinical use nowadays are GnRH agonists for the treatment of prostate and breast cancer (*e.g.*, Buserelin and Nafarelin), GnRH antagonists for the treatment of prostate and breast cancer (*e.g.*, Cetrorelix and Abarelix), and somatostatin agonists for the treatment and diagnosis of GH-producing tumors (*e.g.*, Ocreotide and Lanreotide). In the future, many more peptides will take part in the treatment process against oncology, such as Chlorotoxin and its analogue TM601 (phases I, II, and III clinical trials for diagnosis of glioma), BT1718 (phases I and II for treatment of solid tumors), and P28 (phase I for treatment of various solid tumors)[64].

Drug conjugates is a modern method of using peptides as a tool for drug delivery. They are chemotherapeutic or cytotoxic agents linked to an antibody or a peptide *via* a linker. They provide enhanced function, higher circulation time, and lower off-target toxicity (to healthy tissues)[65]. An example is the conjugation of paclitaxel to a peptide (Angiopep-2) *via* an ester/amide bond. Angiopep-2 goes into the cell *via* transcytosis and crosses the blood-brain barrier, thus facilitating the uptake of the conjugate into the brain for the treatment of patients with solid tumors and brain metastases. The esterase enzyme, which is present in lysosomes, breaks down the ester bond, thus releasing paclitaxel in the brain. In this way, ANG 1005 overcomes the main disadvantage of paclitaxel and gains access into the blood-brain barrier. ANG1005 has been studied in several clinical trials (phase I and phase II) in patients with metastatic brain cancers and the results have shown that it works well against CNS tumors, improves symptoms, and increases survival[66].

Peptide self-assembly is a process in which peptides spontaneously or by a trigger form aggregates. In that form, the transport mechanism provides a higher efficiency of drug loading with better molecule stability and a simultaneous lower ratio of drug loss[67]. The method uses monomers of short amino acid sequences or repeated amino acid sequences that assemble together to form nanostructures. The nanostructure can be made by various building blocks such as dipeptides (the simplest form), surfactant-like peptides, and cyclic peptides[68]. The resultant nanostructure can take the form of nanofibers, nanotubes, micelles, and hydrogels[69-71].

Self-assembly of peptides is divided into spontaneous and trigger types. If the assembly happens in an aqueous solution, it is spontaneous. The peptide molecules that are dissolved in the aqueous solution form non-covalent interactions, such as hydrogen bonding bonds, van der Waals forces, electrostatic, and π-π stacking interactions[69]. If the process of assembly is driven by external factors and does not happen spontaneously, such as temperature, ion concentration, and pH changes, it is called trigger aggregation. The above-mentioned nanostructure can be used for drug delivery, drug stabilization, crossing the blood-brain barrier, neuronal or liver cell regeneration, fibroblast migration, *etc*[69].

NUCLEIC ACIDS

Albeit their central role in governing cell physiology, nucleic acids had not been considered as possible drug candidates until relatively recently when successful protein production was demonstrated upon *in vivo* administration[70]. Since then, a novel class of drugs, referred to as nucleic acid therapeutics, have emerged[71]. Conventional therapeutics generally exhibit a transient effect and exert their action *via* protein targeting. This action mode poses significant disadvantages as only a fraction of human proteins can be targeted by pharmaceutical compounds[72]. Combined with the limitations of conventional therapeutics in oncology discussed above, gene therapy offers a promising approach as a one-time treatment targeting the route of the disease - genetics - while contributing to long-standing therapeutic outcomes with high specificity[73]. Developments in nucleic acid design and chemical modifications have assisted in overcoming stability, toxicity, and immunogenicity issues[74-76], and by further harnessing the power of nanomaterials, nucleic acid therapeutics can be loaded into nanocarriers to formulate DDSs with enhanced pharmacokinetic properties[77].

Due to the arduous nature of the causation and phenotype of cancer, it is evident that nucleic acid-based therapeutics must implement a plethora of strategies *via* different modes of action to target relevant genes and their products in cancer cells or stimulate an immune response against them[78]. Table 3 summarizes the current status of nucleic acid nanomedicines available for cancer treatment. The first strategy implemented in oncology utilizes antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) to target disease-relevant mRNAs and inhibit their translation. ASOs are synthetic oligonucleotides complementary to a gene of interest that bind on the pre-mRNA or mRNA of the target gene, hindering cellular post-transcriptional and translational machinery and eventually leading to altered splicing patterns or gene silencing, respectively[79,80]. Lipid nanoparticle (LNP)-based ASOs are under clinical evaluation to treat leukemia[78,81] and solid tumors[81] *via* targeting Grb2. Furthermore, targeting the anti-apoptotic gene Bcl-2 is also being examined as a possible target in patients with advanced lymphoid malignancies[82].

siRNAs are a class of double-stranded RNA molecules involved in the biological process of RNA interference that regulates gene expression[83]. By administering a siRNA complementary to its target mRNA, this natural process is harnessed to selectively silence genes *via* multiprotein complexes[84]. Activation of the oncogene *KRAS* is a hallmark of pancreatic ductal adenocarcinoma, the most common type of pancreatic cancer[85]. siG12D-LODER is a biodegradable polymer-based system loaded with siRNA against *KRAS*, which has completed phase I and is currently being tested in phase II in combination with chemotherapy to determine treatment efficacy[86-88]. Other delivery systems based on LNPs have also completed or are currently being tested in phase I trials against advanced solid cancers in various modalities targeting tumor proliferation or microenvironment[89-92].

Additional strategies based on small activating RNA molecules aim to upregulate the expression of physiologic master gene regulators[93] and are undergoing pre-clinical development for the treatment of hepatocellular carcinoma[94] and advanced solid tumors[95]. Moreover, since misregulated miRNA expression is another feature of cancer, miRNA mimics are also being developed to mimic endogenous miRNAs and restore physiological expression levels[96,97].

The rise of mRNA vaccines has led to a new era of cancer immunotherapy offering considerable benefits, including increased safety and efficacy with expeditious cost-effective manufacturing pipelines, aiming to elicit an immune response upon exposure to a tumor antigen[98,99]. Several LNP-based mRNA vaccines encoding known tumor-specific antigens are being investigated in early phase clinical trials in patients with HPV-driven squamous cell carcinoma, melanoma, ovarian, pancreatic, lung, and colorectal cancer[100-103]. Technological progress in next-generation sequencing has enormously facilitated the discovery of patient-specific neoantigens, novel epitopes arising from tumor-specific mutations that can be used as a template to generate personalized neoantigen vaccines[104]. Such vaccines are being assessed clinically for the treatment of melanoma and breast cancer[105,106].

CONCLUSION

The field of cancer nanomedicines is rapidly expanding and is expected to revolutionize available treatment options. Nanomaterial-mediated immunomodulation offers a dual aspect of immunomodulation therapies, as they can themselves act as immunomodulatory agents, or they can function as delivery platforms for targeted delivery of other immunomodulating agents[20]. Their unique and tunable properties can be utilized to target the cancer-immunity flow in multiple steps, offering advanced systems that pave a way to reshaping the landscape of clinical cancer treatment. ICIs have launched a new field far beyond CTLA-4 and PD-1. First of all, co-inhibitory signaling pathways, such as HVEM-BTLA and Galectin-9-TIM3, are being studied in cancer and other diseases[107]. Once we learn more about them, we may design rational combinational strategies to concurrently target two or more inhibitory pathways to gain better therapeutic efficacy. Moreover, good results are shown with the combination of immune checkpoint blockade with other immunotherapy regimens to eliminate primary cancer and metastases more effectively. One such strategy has been to combine anti-PD-1/PD-L1 or

Table 3 Summary of nanomedicines based on nucleic acids

Name	Category	Structure	Mode of action	Status
ASO	Inhibition of translation of cancer or angiogenesis associated proteins	Synthetic ssDNA or ssRNA oligos complementary to mRNA of interest	Rnase H mediated mRNA degradation	In clinical trials; LNP-based anti-Grb2 ASOs for leukemia[70] and solid tumors[71]; LNP-based anti-Bcl-2 ASOs for advanced lymphoid malignancies[72]
siRNA	Inhibition of translation of cancer or angiogenesis associated proteins	Synthetic dsRNA oligos complementary to mRNA of interest	Dicer induces cleavage of dsRNA and RNA-induced silencing complex mRNA degradation	In clinical trials; Polymeric anti-KRAS siRNAs for pancreatic ductal adenocarcinoma[78]; LNP based anti-PKN3 siRNAs in patients with advanced solid tumors[79]; LNP based anti-KSP and anti-VEGF-A siRNAs in patients with solid tumors[80,81]; LNP based anti-PLK1 siRNAs in patients with solid tumors[82]
saRNA	Forced exogenous gene expression	Synthetic dsRNA oligos complementary to mRNA of interest	Target gene promoters to induce transcriptional gene activation	In clinical trials; LNP based formulations for treatment of hepatocellular carcinoma[84] and advanced solid tumors[85]
miRNA mimics	Regulation of post-transcriptional mRNA expression	Chemically modified dsRNA molecules designed to mimic endogenous microRNAs	Translational repression and gene silencing	Currently only in basic research[87]
mRNA vaccines	Forced exogenous antigen expression	Synthetic mRNA	Induction of immune response against cancer cells	In clinical trials; LNP-based mRNA vaccines encoding known tumor-specific antigens are being investigated in early phase clinical trials in patients with HPV-driven squamous cell carcinoma[90], melanoma[90], ovarian[92], pancreatic, lung, and colorectal cancer[93]; Personalized vaccines based on patient specific neo-antigens are being assessed clinically for the treatment of melanoma[95] and breast cancer[96]

ASO: Antisense oligonucleotides; saRNA: Small activating RNA; siRNA: Small interfering RNAs; LNP: Lipid nanoparticle.

anti-CTL4 with oncolytic viruses[108,109]. Meanwhile, other types of cancer immunotherapies, including adoptive transfer of CAR T cells, TCR-modified T cells, and cancer vaccines using neo-antigens, have made significant progress in recent years and have shown promise in clinics[110-112]. Future NK cell products will be able to suppress inhibitory signals and tumor proliferation but enhance the activation of the immune system. Evidence of increased NK cell-mediated tumor cell killing has emerged in targeted therapies. To enhance that, nanomedicine approaches immunity with T-cell activation, specific antigen delivery, and the appropriate nanoparticle for the targeting[113]. Nanoparticles will tackle all the obstacles to delivery and engage multiple aspects of the immune system by producing therapeutics to target current and forthcoming diseases[114]. These are only a small portion of the application of nanoparticles with NK cells and their clinical activity because of the heterogeneity of human diseases[115]. These findings, combined with the ability of NK cells to detect immune responses, suggest that NK cells are the keys to the next-generation onco-immunotherapy. In the future days, peptides will play a significant role in the continuous research of cancer therapy and human well-being. Cell-penetrating peptides have the ability to deliver molecules such as drugs, oligonucleotides, and nanoparticles inside cells, without any size restriction[116].

Future research will set the basis for the ideal drug-delivery system, where peptides would reach their target site efficiently without any degradation before and the cargo would be rapidly released and act on the site. Also, the problem of non-selective cellular uptake will be eliminated and thus modern therapy tools for anti-cancer treatment will be created[117,118]. The nanoparticle-mediated delivery of guide RNAs and programmable nucleases such as Cas9 and Cas13 has expanded the portfolio of *in vivo* tissue-specific genome editing tools available for cancer research in pre-clinical models[119-122]. Alongside advancements in nucleic acid drugs, innovative nanoparticle delivery systems will vastly benefit the field by implementing novel delivery systems, such as nanoclews and surface modifications, allowing the manufacturing of sophisticated nanoparticles[123-127]. Despite the numerous nanotherapeutics being clinically scrutinized, cancer nanomedicines often fail to reach their primary endpoint, and the correlation of the drug behavior between animal models and patient cohorts is often inconsistent[128,129]. Therefore, *in silico* models should also be implemented to aid in understanding and predicting biological interactions[130]. Finally, multi-omics data, including but not limited to genomics, epigenomics, transcriptomics, and radiomics, can comprehensively be evaluated and reform the field of personalized nanomedicine by allowing the design of customizable medicines based on the patients' profile[131].

FOOTNOTES

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MINIREVIEWS

Simple approach for the histomolecular diagnosis of central nervous system gliomas based on 2021 World Health Organization Classification

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Abstract

The classification of central nervous system (CNS) glioma went through a sequence of developments, between 2006 and 2021, started with only histological approach then has been aided with a major emphasis on molecular signatures in the 4th and 5th editions of the World Health Organization (WHO). The recent reformation in the 5th edition of the WHO classification has focused more on the molecularly defined entities with better characterized natural histories as well as new tumor types and subtypes in the adult and pediatric populations. These new subclassified entities have been incorporated in the 5th edition after the continuous exploration of new genomic, epigenomic and transcriptomic discovery. Indeed,

the current guidelines of 2021 WHO classification of CNS tumors and European Association of Neuro-Oncology (EANO) exploited the molecular signatures in the diagnostic approach of CNS gliomas. Our current review presents a practical diagnostic approach for diffuse CNS gliomas and circumscribed astrocytomas using histomolecular criteria adopted by the recent WHO classification. We also describe the treatment strategies for these tumors based on EANO guidelines.

Key Words: Central Nervous System glioma; Classification; World Health Organization 2021; European Association of Neuro-Oncology guidelines

Core Tip: Central nervous system (CNS) gliomas went through a sequence of development since 2006. The guidelines of 2021 World Health Organization (WHO) classification of CNS tumors and European Association of Neuro-Oncology (EANO) utilized molecular signatures in the diagnostic approach for CNS gliomas. We herein presents a practical diagnostic approach and the treatment strategies for diffuse CNS gliomas and circumscribed astrocytomas using histomolecular criteria based on the WHO classification.

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INTRODUCTION

Brain tumors are defined as masses derived from various cells originating from the brain (primary tumors) or distally (secondary tumors, most commonly lung, breast, renal, prostate, and skin cancers) that have undergone metastatic spread[1]. The most prevalent primary intracranial tumors are gliomas, with 80% of the population, and World Health Organization (WHO) grade 4 astrocytoma (previously named glioblastoma) is present in nearly one-half of the patients with astrocytoma, with a 95% mortality rate in a 5-year follow-up period irrespective of age and gender[2,3,4]. Gliomas usually present with headache, nausea and vomiting, blurred vision, focal neurological deficit, alteration in sensation, and other manifestations of high intracranial pressure that warrant investigation by neuroimaging, preferably magnetic resonance imaging (MRI) of the brain[5]. In imaging, cystic change, multicentric enhancement, and hemorrhage are commonly observed[6]. Therefore, histopathological examination is considered the gold standard method for diagnosing and grading the tumors.

According to the WHO classification, central nervous system (CNS) gliomas can be classified based on their histological and molecular features[1]. The classification initially includes diffuse and non-diffuse gliomas, and it has undergone significant changes since its establishment to the 4th edition in 2016, aided by molecular signatures[2]. The recent 5th edition of the WHO classification has replaced the entity with type and variant with (subtype) group. In addition, the WHO classification has adopted Arabic numerical over the former Roman numerical grading system for grading brain gliomas for easier reading and to avoid confusion when interpreting pathology reports[7]. However, using the Roman numerical grading system is still considered acceptable. Some of the most important changes in the 5th edition involve the classification of gliomas, differentiating gliomas that occur primarily in adults from those that occur mainly in children[8]. Gliomas are traditionally subclassified into three major categories: diffuse gliomas, pediatric diffuse low- and high-grade gliomas, and circumscribed astrocytic gliomas (Figure 1). In addition, glioneuronal tumors, neuronal tumors, and ependymomas were also included in the classification as separate types[7,8].

Further propositions of the 2021 WHO classification of CNS tumors was adapted by the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy - Not Officially WHO (cIMPACT-NOW), published in 2020/2021. Moreover, they included various molecular genomic studies, including methylation profiling, isocitrate dehydrogenase (*IDH1-2*) codon mutation, and alpha-thalassemia-mental retardation X-chromosome (*ATRX*) mutation. This genomic profiling has affected such tumors' management and treatment modalities[9]. Moreover, three major subtypes of diffuse gliomas have been emphasized, based on molecular genomic signatures, including *IDH*-mutant oligodendroglioma with 1p/19q codeletion, *IDH*-mutant astrocytoma, and *IDH*-wild-type glioblastoma [8].

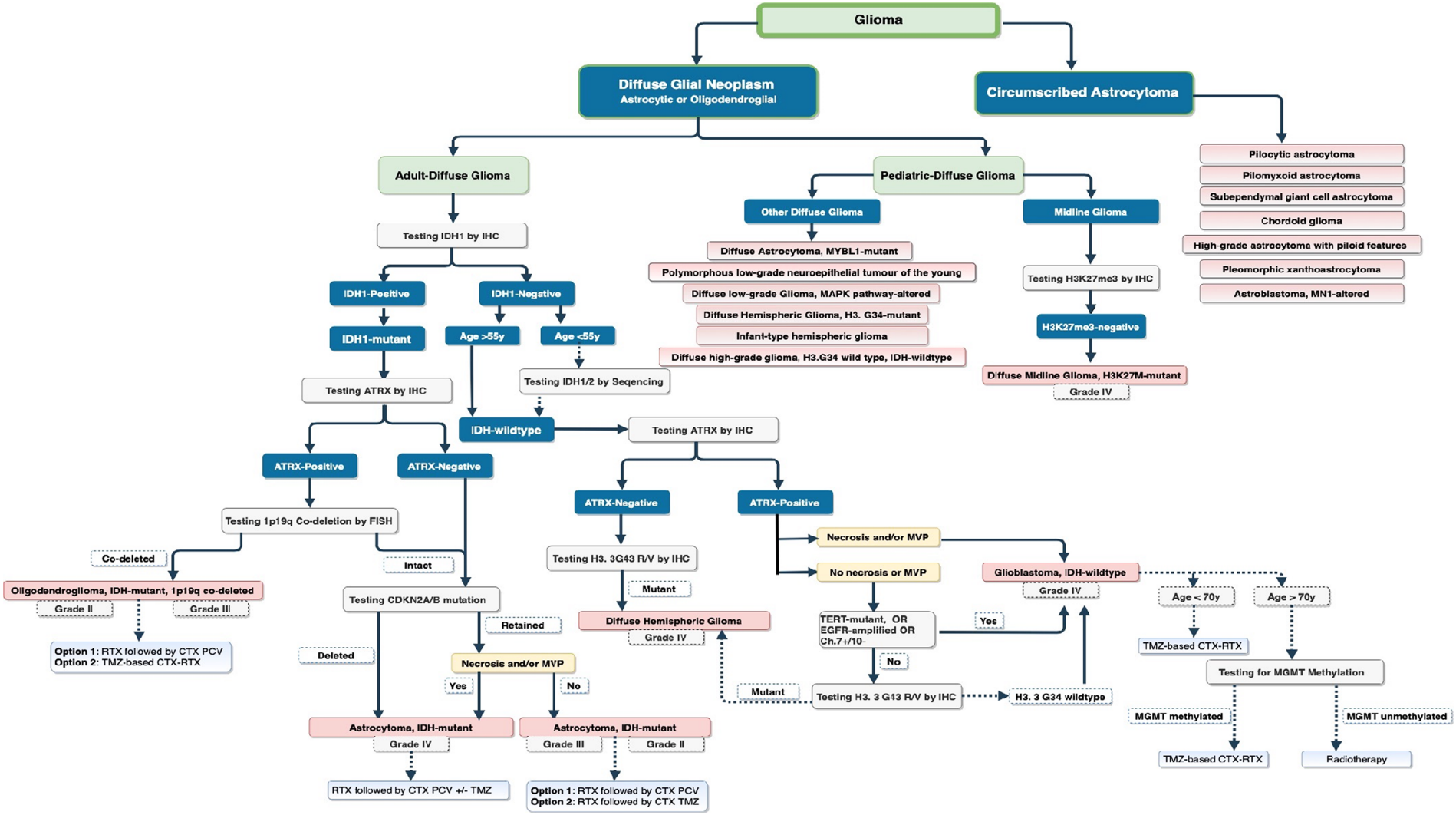

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Figure 1 A simple consensus approach to diagnose diffuse central nervous system gliomas using immunohistochemistry and molecular profiling, aided with the treatment strategy.

In this review, we presented a simple and practical diagnostic approach using histomolecular characteristics to define diffuse CNS gliomas accurately. We also associated the newly published 5th edition of the 2021 WHO classification with the current and updated European Association of Neuro-Oncology (EANO) treatment guidelines.

DISCUSSION

Before 2016, the classification of CNS tumors was established based on histological findings and immunohistochemical tests. Between 2016 and 2021, molecular biomarkers were incorporated into the diagnostic criteria to differentiate CNS tumors into clustered groups[7,9]. The current 5th edition of the 2021 WHO classification does not recommend a specific assessment method to identify molecular alterations unless a distinct tumor subtype is in the differential diagnosis. The cIMPACT-NOW has emphasized the confirmatory diagnosis of astrocytoma with the presence of *IDH* mutation or wild type, *ATRX* loss, *TP53* mutation, and lack of 1p/q19 codeletion[7,10]. In comparison, diffuse gliomas (classified as WHO grades 2 and 3) are either *IDH* wild type or *IDH1*$^{-}$ mutant[11,12]. As a key feature of oligodendroglioma, it, by definition, must harbor an *IDH* mutation and 1p/19q codeletion.

If *IDH1*^{R132H} is immunonegative in astrocytic or oligodendroglial tumors of WHO grades 2 and 3 or in patients aged less than 55 years, IDH1 (132 codon) and/or IDH2 (172 codon) Deoxyribonucleic acid (DNA) sequencing should be performed using the Sanger method or polymerase chain reaction (PCR) [13,14]. Otherwise, IDH1 immunonegative in patients aged greater than 55 years is likely to be IDH-wild type with an incidence rate of < 1% and acts like a high-grade glioma. Meanwhile, IDH1/2 DNA sequencing is preferable for detecting non-canonical mutations[15]. A non-canonical IDH mutation and a loss of ATRX mutation and O(6)-methylguanine-DNA methyltransferase (MGMT) promoter methylation[7] have been associated with a family history of cancer and astrocytoma of the infratentorial region being identified at 80% of the time in multicentric astrocytoma[16].

ATRX mutation should also be tested in all gliomas, whether *IDH1/2* is a mutant or wild type. *ATRX* can be tested by immunohistochemistry (IHC). In oligodendroglioma, positive *ATRX*, negative *TP53*, and *TERT* mutations in *IDH*-mutant tumors are prevalent; nonetheless, 1p19q codeletion using fluorescence in situ hybridization (FISH) should be tested. However, false-positive results are expected to be less than 4% in most cases[17,18]. In both astrocytomas and oligodendrogliomas, the presence of homozygous deletion of *CDKN2A/B* at 9p21 is associated with poor prognosis in such groups, leading to decreased overall survival (OS)[11]. *CDKN2A/B* gene mutation can be tested through next-generation sequencing or the Sanger method if the tumor is *IDH*-mutant and 1p19q is not codeleted[19] (Figure 1). Loss of p16 expression (a marker of CDKNA/B) is associated with poor prognosis in *IDH*-mutant tumors and those with 1p/19q codeletion[20]. *CDKN2A/B* deletion is detected in both the wild-type and mutant *IDH*; however, it has been related to a reduction in OS in wild-type tumors[21-23].

Glioblastomas are routinely diagnosed based on histological findings of microvascular proliferation (MVP) and/or necrosis, and are molecularly defined as either *IDH*-mutant (10%) or *IDH*-wild-type (90%) tumors with significantly different biology and prognoses[8]. The terminology of *IDH*-mutant glioblastoma was omitted from the cIMPACT as they are biologically different from *IDH*-wild-type astrocytomas and were defined as *IDH*-mutant WHO grade 4 astrocytomas. Glioblastoma is referred to as *IDH*-wild-type glioblastoma or astrocytoma. There are no longer *IDH*-mutant glioblastomas. Another new terminology is an astrocytic glioma with *IDH* wild type, but with the presence of a histone 3 (H3) mutation, classified as a WHO grade 4 astrocytoma[9,24] (Figure 1). In glioblastoma, Telomerase reverse transcriptase (*TERT)* promoter mutations, Epidermal growth factor receptor (*EGFR)* mutations, and/or loss of chromosome 10 with gain of chromosome 7 are all prevalent[25]. If one of these features is detected, glioblastoma should be immediately diagnosed regardless of the presence of necrosis and/or MVP[26]. The presence of the H3F3A G34 histone mutation with chromosomal 1q gain distinguishes pediatric diffuse hemispheric glioma from adult diffuse hemispheric glioma. *EGFR*, *TERT*, *CDKNB2A/B*, and Ch10-Ch7+ are usually identified in adults; however, they should be tested for H3F3A gene mutation[27,28]. In comparison, the 2021 WHO classification has included *IDH* wild type and *H3F3A* in diffuse high-grade pediatric astrocytoma (most commonly from the pons in more than two-thirds of the cases, followed by the spinal cord and thalamus)[29].

H3-K27 is another commonly reported histone mutation in midline gliomas, ependymomas, and gangliogliomas[30-32]. Altered mitogen activated protein kinase (*MAPK)* pathway and *H3-K27*-mutant tumors are associated with diffuse low-grade midline gliomas and are associated with prolonged OS of more than 10 years[33]. The astroblastoma tumor is a new entity, *MN1*-altered, with better OS compared to C11Orf95-*RELA* and *BRAF*-positive astrocytic tumors[34]. Four other categories of diffuse astrocytomas were described in the 5th edition of the WHO classification, which included angiocentric gliomas, polymorphous low-grade neuroepithelial tumors of the young, and diffuse low-grade gliomas (*MAP* altered) (Figure 1). Infantile gliomas have a special genetic signature with fusion genes of *MET*, *ROS1*, *ALK*, or *NTRK1/2/3*[35,36].

Guidance to treatment modalities

A positive prognostic factors in diffuse gliomas include young patients, good Karnofsky performance status (KPS), total resection, and *MGMT* promoter methylation[37]. Surgical resection is considered the cornerstone of therapy, with a 5-year survival rate (80%) followed by watchful waiting in low-grade gliomas. However, total resection may result in neurological deficits; thus, awake craniotomy and imaging modalities, such as tractography, may be utilized on a case-by-case basis[38,39]. Postoperatively, MRI can detect residuals, and perfusion studies may detect progression[40]. Therefore, the care plan must be through a multidisciplinary approach with neurooncologists, neuropathologists, and neurosurgeons on board to discuss management modalities.

For low-grade gliomas, such as *IDH*-mutant and 1p/19q codeleted oligodendroglioma (WHO grade 2), careful, a watchful waiting strategy is an option, particularly for totally resected tumors or younger patients (< 40 years) with incomplete tumor resection. However, this would come at a cost: the patient's life, neurological impairments development, and a substantial increase in histological grading over time [41,42]. As a result, disease progression must be monitored with neuroimaging every 2–3 months. According to the National Comprehensive Cancer Network guidelines, patients should be followed up every 2 months with an MRI brain scan, then every 3 months if they have been off therapy for a year [40]. Progression may occur after 4–8 weeks, necessitating a brain MRI. Perfusion studies and spectroscopy can help to differentiate between progression and pseudoprogression, and, in doubt, a multidisciplinary team should be counseled.

Adjuvant radiotherapy

Post-surgical resection radiotherapy is the best therapeutic effect to prevent recurrence or delay the progression of diffuse gliomas (WHO grades 2-4)[43]. The role of radiotherapy is to maintain the control of tumor progression, but it leads to neurotoxicity if used in high doses. Thus, the most used doses are 50-60 Gy administered 3-5 times postoperatively[44]. Choi *et al*[45] found that patients with WHO grade 4 astrocytoma who received 50-60 Gy lived an average of 9 months longer than those who received 45 Gy for 3 months.

Radiotherapy is recommended for all WHO grade 2 gliomas with incomplete resection or for patients aged > 40 years and all WHO grade 3–4 gliomas. Early radiotherapy has been shown to prolong progression-free survival but not OS[46]. Whole-brain radiation therapy is usually not preferable in clinical practice because it is associated with cognitive effects[45]. A follow-up MRI 3-4 weeks after radiotherapy completion is typically performed to monitor disease progression[40]. The use of chemotherapy without radiation remains under investigation but might be an option if radiotherapy is not possible, for example, in patients with large tumors or elderly patients who might not be candidates for radiation.

Chemotherapy

Another adjunct treatment modality for diffuse gliomas is pharmacological treatment, mainly for patients with high-grade gliomas. The most used drug is the alkylating agent temozolomide (TMZ) for its immunomodulation and contribution to tumor-acquiring cell death. TMZ was first discovered in 1987 and has been widely applied as an effective first-line chemotherapeutic agent for treating patients with glioblastoma since the Food and Drug Administration has approved its efficacy in 2005[47,48]. Other drugs used as adjuvants in treating gliomas include nitrosourea, which includes lomustine, carmustine, nimustine, and fotemustine. However, this class of drugs may cause pronounced low platelet count when administered long-term. As a result, patients with oligodendroglioma frequently receive a procarbazine, vincristine, and lomustine (PCV) regimen, as dose-related side effects are more evident in other nitrosourea classes, such as gastrointestinal disorders, decreased cell count, and ototoxicity[48]. Anti-vascular endothelial growth factor antibody (bevacizumab) has also been used as an adjuvant treatment, but it's clear benefit is uncertain[49].

Recent update in glioma treatment

In the new 2021 WHO, cIMPACT-NOW, and EANO guidelines, therapeutic options are targeted at the genomic types of tumors[7,9]. As total surgical resection remains the standard treatment for all CNS gliomas, radiotherapy is still considered the first-line targeted therapy after surgical resection for all WHO grade 2 and 3 oligodendrogliomas or astrocytomas, as mentioned previously[50-52].

In patients with *IDH*-mutant oligodendroglioma (WHO grade 2 or 3) with 1p19q codeletion, aged > 40 years or with no totally resected tumor, and associated with comorbidities, residuals, or recurrence > 15 cm[3], radiotherapy followed by PCV chemotherapy regimen is recommended[51]. According to the two trials (EORTC 26951 and RTOG 9402), the combination of radiotherapy and PCV regimen showed a considerable benefit[52,53].

Radiotherapy followed by chemotherapy is recommended for all patients with *IDH*-mutant WHO grade 2, 3, or 4 astrocytomas, particularly in patients aged > 40 years, with incompletely resected tumors, and associated with neurological deficits[51]. TMZ is often preferred over PCV owing to its safety and ease of administration. However, radiotherapy followed by PCV constitutes the current standard of care for patients with *IDH*-mutant astrocytomas (WHO grade 2)[51]. The RTOG 9802 trial

reported a major prolongation of OS with the addition of PCV to radiotherapy, from 7 to 13 years in patients with WHO grade 2 gliomas who had undergone a subtotal resection or in those aged ≥ 40 years [54]. To prevent functional deficits, diffusion tensor imaging and functional MRI can be utilized[55]. In recurrence, repeat surgery with radiation and chemotherapy (TMZ and nitrosourea) can be considered equally efficient in treatment[9]. For *IDH*-mutant WHO grade 3 astrocytoma, the EORTC 26053 trial of radiotherapy alone, with concomitant or maintenance TMZ, showed a significant prolongation of OS in patients receiving radiotherapy followed by maintenance TMZ[56]. Therefore, TMZ chemotherapy is considered as the standard treatment for tumour progression after surgery and radiotherapy for most patients with *IDH*-mutant gliomas (WHO grade 2 or 3).

Glioblastoma (*IDH*-wild-type grade 4 astrocytoma) is best managed by gross total resection followed by radiotherapy[51]. In non-feasible or nearly total resection cases with age ≥ 70 years, radiotherapy (60 Gy in 30 fractions) or over fractionated radiotherapy (40 Gy in 15 fractions) is preferable to increase OS [9]. Higher survival rates are recorded in younger age groups < 65 years at diagnosis, with a median of up to 40 weeks. However, *TERT* mutation, gain of chromosome 7, and loss of chromosome 10 are associated with poor prognosis[57]. Neurocognitive outcomes can be affected by overfractionated radiotherapy. In patients with good KPS and aged < 70 years, a combination of radiotherapy and chemotherapy (TMZ) is the standard therapy[58]. Combined TMZ with lomustine in early diagnosis may increase OS, particularly in *MGMT*-methylated glioblastomas[59-61]. Hypofractionated radiotherapy is preferable for patients aged ≥ 70 years. The standard-of-care treatment for patients with recurrent glioblastoma has not yet been clarified; treatment is selected based on the prior therapy, patient's age, KPS score, MGMT promoter methylation status, and disease progression. Therefore, surgery and radiotherapy should be considered. Nitrosourea regimens, TMZ, with consideration of bevacizumab are options for pharmacotherapy but have an unconfirmed effect on OS. In patients who did not benefit from adjuvant radiotherapy or had an early symptomatic progression, a second surgery could be considered 6 months after the initial surgery aiming to increase OS[62].

There is a limitation of surgical management in *H3-K27M*-mutant diffuse midline glioma (WHO grade 4) because of its eloquent structures, including the pituitary, thalamus, midbrain, pons, and medulla, and it has a 5-year survival period of < 1%. Radiotherapy is often used but is associated with a poor prognosis. However, in hemispheric glioma, chemoradiotherapy drugs can be used because most of these tumors are *MGMT*-methylated[63].

CONCLUSION

In this review, we presented a simple diagnostic approach to differentiate diffuse CNS gliomas into molecularly defined subtypes using histomolecular features, based on the 5th edition of 2021 WHO classification of CNS tumors. This is to emphasize that molecular profiling is important in the diagnostic classification and grading of diffuse CNS gliomas. We also defined the role of different treatment modalities of surgery, radiotherapy, and pharmacotherapy in the treatment of these molecular defined gliomas. In fact, our review intends to serve as a simple reference for the diagnosis of CNS gliomas for healthcare providers.

FOOTNOTES

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ORIGINAL ARTICLE

Case Control Study

Edmonton Symptom Assessment Scale may reduce medical visits in patients undergoing chemotherapy for breast cancer

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Abstract

BACKGROUND

Adjuvant chemotherapy is recommended in high-risk breast cancer. However, no universally accepted guidelines exist on pre-chemotherapy assessment. In particular, the number and frequency of medical visits vary according to each institution's policy. We hypothesised that the Edmonton Symptom Assessment Scale (ESAS) may have a favourable impact on the pre-treatment assessment in candidates for adjuvant chemotherapy.

AIM

To investigate whether the ESAS can be used to safely reduce the number of medical visits in women with breast cancer undergoing adjuvant chemotherapy.

METHODS

In a retrospectively prospective matched-pair analysis, 100 patients who completed the ESAS questionnaire before administration of adjuvant chemotherapy (ESAS Group) were compared with 100 patients who underwent chemotherapy according to the traditional modality, without ESAS (no-ESAS Group). Patients of the ESAS Group received additional visits before treatment if their ESAS score was > 3. The primary endpoint was the total number of medical visits during the entire duration of the chemotherapy period. The secondary endpoints were the occurrence of severe complications (grade 3-4) and the number of unplanned visits during the chemotherapy period.

RESULTS

The study variables did not statistically differ between patients of the ESAS Group and no-ESAS Group (age $P = 0.880$; breast cancer stage $P = 0.56$; cancer histology $P = 0.415$; tumour size $P = 0.258$; lymph node status $P = 0.883$; immunohistochemical classification $P = 0.754$; type of surgery $P = 0.157$), except for premenopausal status ($P = 0.015$). The study variables did not statistically differ between patients of the ESAS Group and no-ESAS Group regarding age, cancer stage, histology, tumour size, lymph node status, immunohistochemical classification, and type of surgery. Unplanned visits during the entire duration of chemotherapy were 8 in the ESAS Group and 18 in the no-ESAS Group visits ($P = 0.035$). Grade 3-4 toxicity did not differ between the study groups ($P = 0.652$). Forty-eight patients of the ESAS Group received additional visits due to an ESAS score > 3. The mean number of medical visits was 4.38 ± 0.51 in the ESAS Group and 16.18 ± 1.82 in the no-ESAS group ($P < 0.001$). With multivariate analysis, women of the ESAS group were more likely to undergo additional visits for an ESAS score > 3 if they were aged 60 or older, received a mastectomy, or had tumour stage II/III.

CONCLUSION

The ESAS score may safely reduce the number of medical visits in candidates for adjuvant chemotherapy for early breast cancer. Our results suggest that the ESAS score may be used for selecting a group of breast cancer patients for whom it is safe to reduce the number of medical visits in the setting of adjuvant chemotherapy. This may translate into several advantages, such as a more rational utilization of human resources and a possible reduction of coronavirus pandemic infection risk in oncologic patients.

Key Words: Edmonton system assessment scale; Adjuvant chemotherapy; Breast cancer; Medical visits; Patient-reported outcomes

Core Tip: Adjuvant chemotherapy is recommended in high-risk breast cancer. We hypothesized that the Edmonton Symptom Assessment Scale (ESAS) can be used to safely reduce the number of medical visits in women with breast cancer undergoing adjuvant chemotherapy. The main result of this case-matched analysis is that ESAS screening may safely reduce the frequency of medical visits in the setting of AC in patients with breast cancer. This finding may have some advantageous implications in oncological practice, especially in the current scenario, where an increase in coronavirus pandemic cases throughout the world has imposed measures for minimising the risk of infection among patients and health care providers.

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INTRODUCTION

The multidisciplinary treatment of breast cancer has permitted achieving high survival rates over the last 20 years[1-4]. According to current accepted worldwide guidelines, many patients with breast cancer receive recommendation for adjuvant chemotherapy (AC), which continues to be a cornerstone of treatment for high-risk patients. In fact, AC has been linked to a reduced risk of developing locoregional and systemic recurrences, as well as to increased overall survival in some subgroups of patients who have undergone surgery for breast cancer[5-7]. However, it is known that toxicity of chemotherapy regimens can expose patients to adverse effects, unplanned medical visits, or hospitalisation[4,7,8].

There are no globally standardised guidelines that regulate the pre-treatment assessment of candidates for AC. While it is established that administration of chemotherapy drugs should be done by oncology nurses under the supervision of a medical oncologist, some aspects of the treatment vary according to each institution's policy. In common practice, prior to every session of chemotherapy patients are evaluated during a medical visit. A pre-chemotherapy medical visit before every cycle of AC represents a time- and resource-demanding practice, especially in high-volume centres. The Edmonton Symptom Assessment Scale (ESAS) is a useful and simple tool for evaluating patients

undergoing therapy for cancer. The ESAS consists of a questionnaire developed to rate the intensity of nine common symptoms experienced by patients with cancer[9-11]. We hypothesised that the ESAS can be used to safely reduce the number of medical visits in women undergoing AC for breast cancer. Therefore, we conducted a prospective matched-pair analysis to evaluate the impact of the ESAS in this subgroup of patients.

MATERIALS AND METHODS

Study population

Patients receiving treatment for breast cancer were prospectively registered in an institutional board-registered database at the Breast Unit of the University Hospital of Sassari (Italy). According to the institutional policy, all patient cases were presented in a weekly multidisciplinary meeting, in which preoperative and postoperative management was discussed. After metastatic work up, each patient received neoadjuvant chemotherapy or upfront surgery (mastectomy or breast-conserving surgery [BCS] and sentinel node biopsy with or without axillary lymphadenectomy, according to the status of the sentinel node). Radiotherapy was given after BCS and in selected high-risk patients after mastectomy, in accordance with current guidelines. Adjuvant endocrine therapy was administered for 5 years to all women with oestrogen receptor-positive breast cancer after the completion of chemotherapy. Trastuzumab was recommended for women with HER2-positive tumours (immunohistochemistry 3+) for a total duration of 1 year. For the purpose of this study, we asked our database for patients who had undergone AC for Stages I-III breast cancer from January 2018 to November 2021. To be eligible for the present study, patients had to fulfil the following criteria: female gender, age ranging from 18 to 75 years old, diagnosis of unilateral or bilateral operable primary breast carcinoma without distant metastases, and sequential chemotherapy comprising epirubicin and cyclophosphamide followed by taxane. Exclusion criteria were neoadjuvant chemotherapy, metastatic disease, recurrent breast cancer, pregnancy, or lactation.

Study design

The study was approved by the Institutional Board of the AOU of Sassari. From January 2020, patients scheduled for AC were offered to participate in a programme where the ESAS was provided during the chemotherapy treatment period. All patients signed a written consent form before entering the ESAS programme. In a case-matched analysis, data from 100 patients taking the ESAS (the ESAS Group) in the period January 2020 to November 2021 were compared with data of 100 patients who underwent AC according to the traditional modality, without the ESAS (the no-ESAS Group) during the previous period (January 2016-December 2019). All patients of the study were scheduled to receive the following sequential regimen: Four cycles of epirubicin and cyclophosphamide followed by 12 cycles of paclitaxel (4EC-12T). Patients of the ESAS Group received the ESAS questionnaire translated into Italian before every cycle of AC; a medical visit was scheduled before the first cycle of epirubicin and cyclophosphamide, before the first cycle of taxane, and before the last cycle of taxane[12,13]. Therefore, each patient of the ESAS Group was scheduled to receive a total of three medical visits for the entire AC duration; an additional medical visit before each chemotherapy session was carried out according to the ESAS score (specifically in the all cases where the ESAS score was > 3). Patients of the no-ESAS Group received a medical visit before every cycle of AC. Therefore, each patient of the no-ESAS Group was scheduled to receive a total of 16 medical visits for the entire AC duration (Figure 1).

The matching variables included age and breast cancer stage. We decided to perform a case-matched analysis to obtain a more homogenous control group, and to minimise differences between groups due to the extent of disease. For all patients, the following data were extracted: Age, year of diagnosis, menopausal status, tumour size, histological type, axillary lymph nodes status, immunohistochemical classification, type of upfront surgery, and breast cancer stage. In the ESAS Group, patients who needed additional medical visits based on the ESAS score > 3, were identified. In both study groups (ESAS and no-ESAS) percentage of patients requiring unplanned medical visits (defined as visits for problems related to the surgical procedure or chemotherapy-related side effects), the number of unplanned medical visits, and grade 3-4 adverse effects during chemotherapy treatment, were calculated.

Study endpoints and statistical analysis

The primary endpoint was the total number of medical visits per patient during the entire duration of AC. The secondary endpoints were the occurrence of severe complications (grade 3-4) during the administration of AC and the number of unplanned visits during the cycles of chemotherapy. In addition, independent factors associated with the likelihood of receiving additional visits due to an ESAS score > 3 were analysed. Quantitative variables are presented as a mean; qualitative variables are presented as absolute numbers and percentages. Categorical variables were compared by the chi-square test or Fisher's exact test where appropriate. Continuous variables were assessed by Student's *t*-test or the Mann-Whitney U test. A P value < 0.05 was used as the threshold for statistical significance. In the ESAS Group, the likelihood of receiving additional visits on the basis of an ESAS score > 3 was analysed

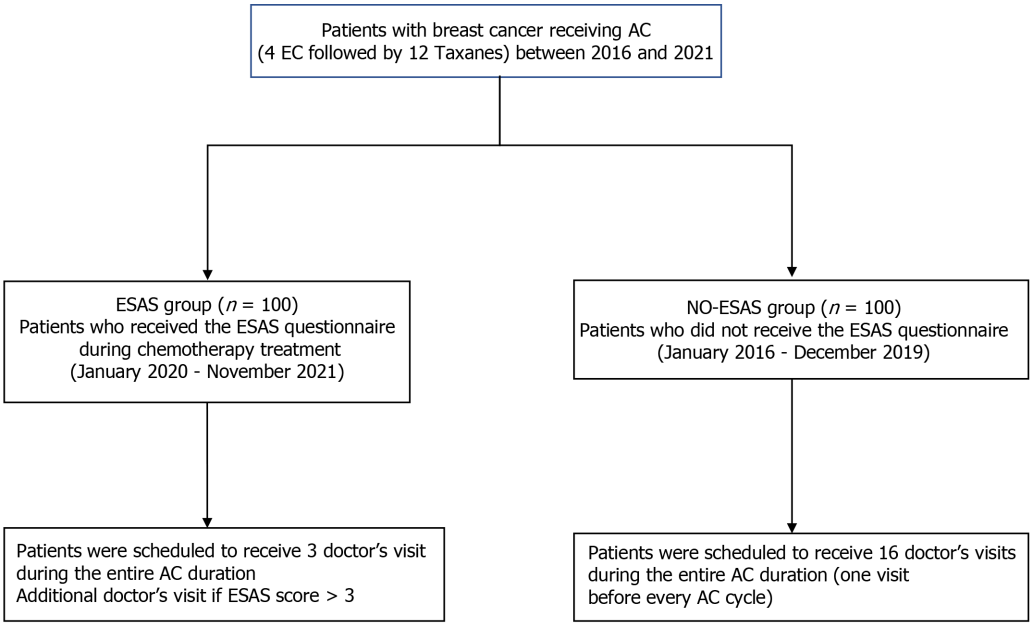

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Figure 1 Study design. AC: Adjuvant chemotherapy; ESAS: Edmonton Symptom Assessment Scale.

with a multivariable logistic regression model. Each factor was dichotomised to a binary variable: Age (≤ 60 years *vs* > 60 years), type of surgery (BCS *vs* mastectomy), immunohistochemical classification (luminal *vs* non-luminal), and tumour stage (stage I *vs* stage II/III). Covariates were chosen on the basis of clinical significance. For each dichotomous variable, a reference category was chosen, generally the majority category, and compared with the other category. The odds ratio (OR) in each category *vs* the reference category was estimated. The goodness of fit of the model was assessed by the Hosmer-Lemeshow test, and $P > 0.05$ indicated a good fit. Statistical analyses were conducted by using SPSS Statistics 20 (IBM Corp., Armond, NY, United States).

RESULTS

Patient and tumour characteristics

Demographic and tumour characteristics are presented in Table 1. The mean age at diagnosis was 57.2 years. Tumour size was ≤ 2 cm in 48% of patients and > 2 cm in 52%. The most common histology was invasive ductal carcinoma (86%), followed by lobular invasive carcinoma (14%). Thirty-five per cent of patients were premenopausal. The majority of patients had tumours of stage II/III (60%). Fifty-three per cent of patients underwent BCS, while 47% underwent a mastectomy. Axillary lymph node status was positive in 37% of cases and negative in 63%. Regarding the immunohistochemical classification, the most frequent subtype was HER2-enriched (54%), followed by luminal B (23%), triple-negative (13%), and luminal A (10%) tumours. The study variables did not differ significantly between patients of the ESAS Group and the no-ESAS Group (mean age $P = 0.524$; age ≤ 60 years $P = 0.880$; breast cancer stage $P = 0.56$; cancer histology $P = 0.415$; tumour size $P = 0.258$; axillary lymph node status $P = 0.883$; immunohistochemical classification $P = 0.754$; type of surgery $P = 0.157$), except for premenopausal status, which was more frequent in the ESAS Group ($P = 0.015$). There were there 8 additional unplanned visits for 6 patients in the ESAS Group, and 18 additional visits for 12 patients in the no-ESAS Group ($P = 0.035$) Six patients of the ESAS Group and 12 of the no-ESAS Group needed one or more unplanned visit during the AC duration, for a total of 8 and 18 visits, respectively ($P = 0.057$). Grade 3-4 toxicity occurred in two and three patients of the ESAS Group and the no-ESAS Group, respectively ($P = 0.652$). Forty-eight patients of the ESAS Group received an additional visit due to an ESAS score > 3. Globally, the mean number of medical visits was 4.38 ± 0.51 in the ESAS Group and 16.18 ± 1.82 in the no-ESAS Group ($P < 0.001$) (Table 2).

Based on multivariate analysis, women of the ESAS Group were more likely to undergo additional visits before chemotherapy for an ESAS score > 3 if they were aged > 60 years, received a mastectomy, or had tumour stage II/III (Table 3). We did not find any association between additional visits and immunohistochemical tumour classification or lymph node status. Age > 60 years was the strongest predictor of receiving additional medical visits before chemotherapy (OR 4.93, 95% confidence interval 1.26-19.25).

Table 1 Clinicopathological characteristics of the study population

Characteristic	Group A (ESAS) (*n* = 100)	Group B (No-ESAS) (*n* = 100)	*P* value
Age (mean ± SD)	57.7 ± 11.5	56.6 ± 12.4	0.524
Age groups, *n* (%)			0.880
≤ 60 yr	64 (64)	62 (62)	
> 60 yr	36 (36)	38 (38)	
Premenopausal status	51 (51)	34 (34)	0.015
Breast cancer stage, *n* (%)			
I	44 (44)	36 (36)	0.506
II	28 (28)	31 (31)	
III	28 (28)	33 (33)	
Cancer histology, *n* (%)			
Ductal	84 (84)	88 (88)	0.415
Lobular	16 (16)	12 (12)	
Tumour size (mean ± SD) , *n* (%)			0.258
≤ 2 cm	52 (52)	44 (44)	
> 2 cm	48 (48)	56 (56)	
Lymph node status, *n* (%)			0.883
N0	66 (66)	59 (59)	
N+	34 (34)	41 (41)	
Himmunohistochemical classification, *n* (%)			0.754
Luminal A	12 (12)	8 (8)	
Luminal B	21 (21)	25 (25)	
HER2 positive	55 (55)	54 (54)	
TNBC	12(12)	13 (13)	
Type of surgery, *n* (%)			0.157
BCS	58 (58)	48 (48)	
Mastectomy	42 (42)	52 (52)	

ESAS: Edmonton Symptom Assessment Scale; BCS: Breast-conserving surgery.

DISCUSSION

Various chemotherapy regimens, which can be associated with either minor or major toxicity, are commonly used for AC in patients undergoing surgery for breast cancer[4,14]. However, no recognised guidelines exist regarding some aspects of this important part of the multidisciplinary treatment. The main result of this case-matched analysis is that ESAS screening may safely reduce the frequency of medical visits in the setting of AC in patients with breast cancer. This finding may have some advantageous implications in oncological practice, especially in the current scenario, where an increase in coronavirus pandemic 2019 (COVID-19) cases throughout the world has imposed measures for minimising the risk of infection among patients and health care providers.

Pre-chemotherapy assessment varies among oncology services. On a general basis, during the medical visit before chemotherapy, relevant information to manage any possible treatment side effect are collected, and a physical examination might be carried out. In the present study, we have used the ESAS score as a patient-reported outcomes tool. The ESAS is one of the first multidimensional assessment tools that has been used in clinical practice. The scale was created for the clinical assessment of the increase and modification of symptoms in patients with advanced cancers admitted to palliative care units[11,15,16]. The ESAS score has subsequently been validated in various studies and used as a tool for the detection of symptoms divided by clusters, favouring the implementation of interventions for symptom management[17]. In patients with breast cancer, correct symptom assessment and

Table 2 Outcomes of interest during the chemotherapy treatment in the study population

Variable	Group A (ESAS)	Group B (No ESAS)
N of doctor visit scheduled for each patient	3	16
Total No. of scheduled doctor visits	300	1600
N of patients requiring adjunctive visit on the bases of ESAS score > 3	48	-
N of adjunctive visits on the bases of ESAS score > 3	130	-
N of patients requiring unplanned doctor visits	6	12
N of unplanned doctor visit	8	18
Effective total No. of doctor visits[a]	438	1618
N of visits for each patient (mean ± SD)[b]	4.38 ± 0.51	16.18 ± 1.82
Adverse effects during chemotherapy treatment	2	3

[a]$P < 0.001$.
[b]$P < 0.001$. ESAS: Edmonton Symptom Assessment Scale.

Table 3 Multivariate logistic regression for factors associated with the need of additional medical visits before chemotherapy in patients the Edmonton Symptom Assessment Scale Group (Edmonton Symptom Assessment Scale score > 3)

Variable	Odds ratio	St. Error	Z-score	95%CI	*P* value
Age					
> 60 (*n* = 36)	4.93	0.695	1.596	1.26-19.25	0.022[a]
≤ 60 (*n* = 64)	Ref.				
Lymph node status					
Positive (*n* = 44)	0.50	0.662	0.691	0.13-1.83	0.297
Negative (*n* = 66)	Ref.				
Type of surgery					
Mastectomy (*n* = 42)	0.15	0.726	-1.895	0.03-0.62	0.009[b]
BCS (*n* = 58)	Ref.				
IHC classification					
Luminal (*n* = 33)	1.96	0.699	0.674	0.49-7.73	0.335
Non-Luminal (*n* = 67)	Ref.				
Tumour stage					
I (*n* = 44)	0.86	0.880	0.149	1.12-35.44	0.036[c]
II/III (*n* = 56)	Ref.				

[a]$P < 0.05$.
[b]$P < 0.05$.
[c]$P < 0.05$. BCS: Breast-conserving surgery.

management still represent a challenge for medical oncologists[18]. Specifically, in the early setting of the disease, the correct assessment and management of symptoms is essential to improve quality of life and patient adherence to treatments and, therefore, the effectiveness of adjuvant therapies.

Several studies have explored the role of the ESAS to predict patient-related outcomes in patients with breast cancer, especially in the setting of advanced disease[19]. In a recent review including nine articles, the authors reported that the ESAS score is a promising tool for predictive modelling of time to death in patients with breast cancer receiving palliative care[19]. However, few studies have investigated the role of the ESAS in the setting of breast cancer. In patients with non-metastatic breast cancer who received radiotherapy, the ESAS score has been used to identify significant symptoms linked to a worse overall quality of life[20].

In the series described herein, we found that the patients who completed the ESAS questionnaire received significantly fewer medical visits during chemotherapy period compared with patients of the control group. In the series described herein, we found that the use of the ESAS questionnaire allowed to identify patients who required additional medical visits before a chemotherapy cycle. To note, the reduction in the number of scheduled visits based on the ESAS score, did not affect the occurrence of complications from chemotherapy, and was associated to a reduced number of unplanned medical visits. In fact, patients of the ESAS Group were scheduled to receive only three visits; additional visits were deemed necessary only when the ESAS score was > 3. These findings are consistent with the experience of Barbera *et al*[14], who demonstrated that screening with the ESAS was associated with decreased emergency department visits by patients with breast cancer receiving AC. It has been suggested that screening of routine symptoms, using tailored patient-reported outcomes tools, could be useful for improving patient/physician communication, helping to monitor the treatment response and identifying unrecognised problems[20-23].

In this study, we hypothesised that the ESAS score in the setting of AC would be able to safely reduce the number of medical visits. We used the occurrence of grade 3-4 chemotherapy toxicity as a surrogate of safety; this measure did not differ between the two study groups. The need for medical visits in patients undergoing AC for breast cancer depends on many tumour- and patient-related factors[4,14]. In our experience, patients aged > 60 years had a fourfold increased risk of receiving additional visits based on the ESAS score, reflecting the importance of patient age regarding anticancer treatments. Of note, the number of unplanned medical visits due to acute toxicity experienced by patients was lower in the ESAS Group. In another study involving a cohort of 2541 patients with stage I-III breast cancer, women undergoing chemotherapy for breast cancer screened with the ESAS had a 43% lower rate of emergency department visits than those who were not screened with the ESAS[14].

Medical visits for pre-chemotherapy assessment represent a significant burden on the oncological care system. There are several potential advantages of reducing the number of medical visits in patients receiving AC. First, although we did not calculate the time spent on every visit, we can assume that the reduced number of medical visits does translate to a significant sparing of time in oncology departments; hence, oncologists and nurses may spend their time on other clinical activities. This may have important implications especially in high-volume oncology centres. Second, the ESAS score permits patients to take an active role in deciding the course of their AC treatment. Generally, patient-reported outcomes have been gaining importance for describing subjective symptoms and improving quality of life[4,23,24]. Studies have compared the description of toxicity and adverse effects by using patient-related-outcome tools in comparison with physician-reported findings. A possible underestimation of the incidence and the entity of symptoms reported by physicians has been evidenced[25,26]. Baratelli *et al* demonstrated, in a cohort of 211 patients receiving active anticancer treatment, that these tools produced high patient satisfaction and a significant quality-of-life improvement, compared with the traditional modality of a medical visit[23]. Third, in the current scenario, where contact restrictions are encouraged, use of the ESAS questionnaire may reduce the risk of COVID-19 infections among oncologic patients. In fact, the decrease in medical visits could reduce both personal contacts and the duration of stay in oncology units among patients with chemotherapy-induced immunosuppression. At the time of writing, the world is experiencing a new wave of the pandemic due to the delta and omicron variants of severe acute respiratory syndrome coronavirus 2.

Several studies have investigated the role of the ESAS score on quality-of-life perception, supportive care needs and symptom assessment in patients with cancer; however, to the best of our knowledge, this is the first study focussing on its impact on medical visits in the setting of AC. We recognise that this work has some limitations, the main one being the small sample size. Furthermore, we arbitrarily decided to set the ESAS score cut-off point for patients to receive additional medical visits for AC administration as 3. Regarding this matter, the optimal cut-off points for the symptoms and quality indicators of the ESAS remain ill defined[27,28].

CONCLUSION

In summary, our work provides evidence that the use of the ESAS score may safely reduce the number of medical visits in patients undergoing AC. Moreover, it implies that ESAS may help to identify patients who do not need to visit a doctor during each course of chemotherapy, as well as to identify a group of patients with a high risk of complications in whom a treatment adjustment is needed. This may result in several advantages for both patients and health care providers, especially in the current COVID-19 pandemic. Additional studies are needed to gain new insights into the role of patient-reported outcome strategies in the management of AC in the setting of breast cancer.

ARTICLE HIGHLIGHTS

Research background

Adjuvant chemotherapy (AC) represents a fundamental part of multidisciplinary treatment of women with high-risk breast cancer, since it has been associated to a reduced risk of developing cancer recurrence, as well as to an increased survival. However, no standardised guidelines that regulate the pre-treatment assessment of patients candidates for AC exist. In common practice, a pre-chemotherapy medical visit before every cycle of AC is scheduled, and this represents a time- and resource-demanding practice.

Research motivation

Accurate use of the Edmonton Symptom Assessment Scale (ESAS) may lead to identify patients who do not need to visit a doctor during each course of AC.

Research objectives

To evaluate the value of the ESAS in safely reduce the number of medical visits prior adjuvant chemotherapy.

Research methods

One-hundred breast cancer women candidates to AC were administered the ESAS score (ESAS Group), and were scheduled to receive a total of three medical visits for the entire AC duration. They were prospectively compared to a to a matched-pair group of 100 patients who received adjuvant chemotherapy without ESAS (no-ESAS Group) and were scheduled to receive 16 medical visits for the entire AC duration. Study endpoints were the number of medical visits, occurrence of severe complications, and the number of unplanned visits.

Research results

The mean number of medical visits was 4.38 ± 0.51 in the ESAS Group and 16.18 ± 1.82 in the no-ESAS group ($P < 0.001$). Unplanned visits during the entire duration of chemotherapy were 8 in the ESAS Group and 18 in the no-ESAS Group visits ($P = 0.035$). Grade 3-4 toxicity did not differ between the study groups ($P = 0.652$). Forty-eight patients of the ESAS Group received additional visits due to an ESAS score > 3. With multivariate analysis, women of the ESAS group were more likely to undergo additional visits for an ESAS score > 3 if they were aged 60 or older, received a mastectomy, or had tumour stage II/III.

Research conclusions

Our results suggest that the ESAS score may be used for selecting a group of breast cancer patients for whom it is safe to reduce the number of medical visits in the setting of AC. This may permit a more rational utilization of human resources and a possible reduction of coronavirus pandemic 2019 infection risk in oncologic patients.

Research perspectives

Additional studies are needed to gain new insights into the role of patient-reported outcome strategies in the management of AC in the setting of breast cancer.

FOOTNOTES

Author contributions: Sanna V and Fancellu A designed the study, supervised, wrote and edited the final version; Deiana G, Ninniri C and Alicicco MG provided original data, collected variables, and analysed data; Fedele *P* and Santoro AN provided technical support, figures, tables, and reviewed the manuscript; Pazzola A envisioned the study, and edited the final manuscript.

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ORIGINAL ARTICLE

Case Control Study

Awareness, knowledge, and attitudes towards sun protection among patients with melanoma and atypical mole syndrome

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Abstract

BACKGROUND

Patients with atypical mole syndrome (AMS) have a 3- to 20-fold higher risk of developing malignant melanoma (MM) than individuals without. The most modifiable risk factor for developing MM is the ongoing ultraviolet exposure.

AIM

To assess awareness, knowledge, and attitudes towards sun protection among patients with MM and AMS.

METHODS

From January 2020 till December 2021, a written survey was administered to patients with MM and AMS and a control group who attended a specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion in Heraklion, Crete, Greece. Demographic data and photoprotective practices, knowledge, and perceived barriers were collected. Relevant statistical analyses were performed using SPSS IBM 25.

RESULTS

In total, 121 subjects consented and participated in the survey. Their mean age was 43.92 ± 12.55 years. There were 66 (54.4%) females and 55 (45.4%) males. Forty-seven (38.8%) patients had AMS, 26 (21.5%) had a past medical history of

MM, and 48 (39.7%) attended the clinic for a full skin checkup for their naevi without having AMS or MM. Although 104 (86%) participants reported using sunscreen with the majority of them (59/121 = 48.8%) wearing sunscreen with a sun protection factor of > 50, only 22 (18.2%) patients did so every day and only 20 (16.5%) all year round. Approximately 74.4% of patients recalled having received advice on how to protect their skin from sunlight, and 73% were interested in receiving education about sun protection. The most mentioned barriers in photoprotection were concerns over adequate vitamin D and lack of time.

CONCLUSION

Despite mentioning having received adequate education in photoprotection, adherence to photoprotection practices is suboptimal in patients with MM and AMS.

Key Words: Atypical mole syndrome; Dysplastic naevi; Malignant melanoma; Photoprotection; Skin cancer

Core Tip: There are no previous studies assessing awareness, knowledge, and attitudes towards sun protection among patients with malignant melanoma (MM) and atypical mole syndrome (AMS). Our study highlights the importance to raise awareness regarding photoprotection in patients with MM and AMS to prevent skin cancer.

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INTRODUCTION

The term atypical mole syndrome (AMS) refers to people who have multiple naevi (> 100), including some naevi larger than 8 mm in diameter with atypical features[1,2]. Patients with AMS have a 3-20 times higher risk of developing malignant melanoma (MM) than individuals without[3-6]. The most modifiable risk factor for developing MM is ongoing ultraviolet (UV) exposure[7]. Eliminating UV exposure *via* photoprotective practices is an important strategy for reducing MM risk in patients with AMS[8-10].

Through the implementation of a written survey, our aim for this study was to assess awareness, knowledge, and attitudes toward sun protection among patients with MM, those with AMS, and a control group who attended a specialist mole clinic at the dermatology department of a tertiary hospital in Greece.

MATERIALS AND METHODS

From January 2020 through December 2021, we administered a written survey to patients who attended a specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion in Heraklion, Crete, Greece. Having approached 140 patients, we obtained consent from 121 patients (a response rate of 121/140 = 86.42%). The participants completed the surveys in person, and we included all the data in our analysis.

The specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion is a dedicated clinic for patients at high risk of developing skin cancer, such as those who have a past medical history (PMH) of MM, non-melanoma skin cancer, or AMS or who have received immunosuppression (*e.g.*, transplant patients). All these patients undergo annual or biannual full skin checkups and receive photoprotection counselling.

The study was approved by the Ethics Committee of the University Hospital and all participants gave consent for inclusion in the study.

Survey contents

The written survey that we administered included basic demographic data, Fitzpatrick skin phototypes, medical histories, comorbidities, and collected information regarding awareness and knowledge of

Table 1 Demographic and clinical characteristics of 121 patients who were included in our study

	Patients with a PMH of MM, *n* = 26/121 (21.5%)	Patients with AMS, *n* = 47/121 (38.8%)	Control group, *n* = 48/121 (39.7%)	All participants, *n* = 121	*P* value
Mean age (± SD)	45.65 (± 12.61)	43.21 (± 12.61)	43.67 (± 12.65)	43.92 (± 12.55)	0.88
Gender, *n* (%)					0.39
Male	10/26 (38.5)	25/47 (53.2)	20/48 (41.7)	55/121 (45.5)	
Female	16/26 (61.5)	22/47 (46.8)	28/48 (58.3)	66/121 (54.5)	
Employment Status, *n* (%)					0.40
Student	2/26 (7.7)	3/47 (6.4)	2/48 (4.2)	7/121 (5.8)	
Employed	16/26 (61.5)	36/47 (76.6)	33/48 (68.8)	85/121 (70.2)	
Unemployed	2/26 (7.7)	4/47 (8.5)	7/48 (14.6)	13/121 (10.7)	
Retired	5/26 (19.2)	3/47 (6.4)	3/48 (6.3)	11/121 (9.1)	
Housewife	1/26 (3.8)	1/47 (2.1)	3/48 (6.3)	5/121 (4.1)	
Educational level, *n* (%)					0.61
Elementary school	9/26 (34.6)	1/47 (2.1)	3/48 (6.3)	4/121 (3.3)	
High school	0/26 (0)	20/47 (42.6)	21/48 (43.8)	50/121 (41.3)	
Technical studies	6/26 (23.1)	8/47 (17)	5/48 (10.4)	19/121 (15.7)	
University level	11/26 (42.3)	18/47 (38.3)	19/48 (39.6)	48/121 (39.7)	
Fitzpatrick skin phototype, *n* (%)					0.81
Skin type I (Always burns, does not tan)	0/26 (0)	2/47 (4.3)	2/48 (4.2)	4/121 (3.3)	
Skin type II (Burns easily, tans poorly)	10/26 (38.5)	14/47 (29.8)	15/48 (31.3)	39/121 (32.2)	
Skin type III (Tans after initial burn)	14/26 (53.8)	24/47 (51.1)	22/48 (45.8)	60/121 (49.6)	
Skin type IV (Burns minimally, tans easily)	2/26 (7.7)	7/47 (14.9)	9/48 (18.8)	18/121 (14.9)	
BMI (± SD)	25.07 (± 4.06)	26.92 (± 5.12)	25.58 (± 5.20)	25.99 (± 4.96)	0.281
Eye colour, *n* (%)					0.466
Dark	1/26 (3.8)	2/47 (4.3)	5/48 (10.4)	8/121 (6.6)	
Brown	16/26 (61.5)	31/47 (66)	31/48 (64.4)	78/121 (64.5)	
Blue	3/26 (11.5)	9/47 (19.1)	5/48 (10.4)	17/121 (14)	

Green	6/26 (23.1)	5/47 (10.6)	7/48 (14.6)	18/121 (18)	
Natural hair color, *n* (%)					0.649
Red	0/26 (0)	1/47 (2.1)	0/48 (0)	1/121 (0.8)	
Blond	5/26 (19.2)	6/47 (12.8)	8/48 (16.7)	19/121 (15.7)	
Brown	16/26 (61.5)	34/47 (72.3)	28/48 (58.3)	78/121 (64.5)	
Black	5/26 (19.2)	6/47 (12.8)	12/48 (25)	23/121 (19)	
Number of naevi, *n* (%)					0.000
< 25 naevi	9/26 (34.6)	0/47 (0)	23/48 (47.9)	32/121 (26.4)	
25-50 naevi	8/26 (30.8)	2/47 (4.3)	12/48 (25)	22/121 (18.2)	
50-100 naevi	3/26 (11.5)	11/47 (23.4)	7/48 (14.6)	21/121 (17.4)	
100 naevi	6/26 (23.1)	34/47 (72.3)	6/48 (12.5)	46/121 (38)	
Smoking status, *n* (%)					
Current smoker	8/26 (30.8)	15/47 (31.9)	9/48 (18.8)	32/121 (26.4)	0.198
No smoker	15/26 (57.7)	25/47 (53.2)	28/48 (58.3)	68/121 (56.2)	
Ex-smoker	3/26 (11.5)	7/47 (14.9)	11/48 (22.9)	21/121 (17.4)	
Sunburn before the age of 18, *n* (%)					0.000
No	9/26 (34.6)	30/47 (63.8)	40/48 (83.3)	79/121 (65.3)	
Yes	17/26 (65.4)	17/47 (36.2)	8/48 (16.7)	42/121 (34.7)	
Leisure sun exposure, *n* (%)					0.393
No	17/26 (65.4)	31/47 (66)	38/48 (79.2)	86/121 (71.1)	
Yes	9/26 (34.6)	16/47 (34)	10/48 (20.8)	35/121 (28.9)	
Occupational sun exposure, *n* (%)					0.35
No	19/26 (73.1)	27/47 (57.4)	30/48 (62.5)	76/121 (62.8)	
Yes	7/26 (26.9)	20/47 (42.6)	18/48 (37.5)	45/121 (37.2)	
Significant time spent outdoors, *n* (%)					0.356
No	11/26 (42.3)	24/47 (51.1)	28/48 (58.3)	63/121 (52.1)	
Yes	15/26 (57.7)	23/47 (48.9)	20/48 (42.7)	58/121 (47.9)	
Mean weeks of vacation spent before the age of 10 (± SD)	7.35 ± 5.61	6.87 ± 5	7.42 ± 4.36	7.19 ± 4.86	0.7444

Mean weeks of vacation spent before from the age of 11 till 18 (± SD)	6.12 ± 4.27	6.38 ± 4.44	6.94 ± 4.20	6.55 ± 4.29	0.740
Mean weeks of vacation spent after the age of 18 (± SD)	3.92 ± 2.1	4.02 ± 2.77	4.98 ± 4.35	4.39 ± 3.40	0.806

AMS: Atypical mole syndrome; PMH: Past medical history; MM: Malignant melanoma; SD: Standard deviation; BMI: Body mass index.

photoprotection measures and current sun-protective practices. The participants were asked to report any difficulties that discouraged them from practicing photoprotective measures. We administered the survey to patients after they received counseling on photoprotection from the dermatology outpatient mole clinic.

Statistical analysis

Descriptive statistics, ANOVA, Kruskal-Wallis test, *t* tests, and Pearson correlation tests were performed using SPSS version 25.0.

RESULTS

Demographic data

Of the 140 patients that we approached who attended the specialist mole clinic at the Dermatology Department of the University Hospital of Heraklion in Heraklion, Crete, Greece from January 2020 until December 2021, 121 consented to and participated in the study, making our response rate be 121/140. Their mean age was 43.92 ± 12.55 years. There were 66 (54.4%) females and 55 (45.4%) males. Forty-seven (38.8%) patients had AMS, 26 (21.5%) had a PMH of MM, and 48 (39.7%) attended the clinic for a full skin checkup for their naevi without having AMS or MM. The main demographic and clinical characteristics of these 121 patients are summarized in Table 1. There were no statistical differences among the three groups of patients for the following demographics and clinical characteristics: Age; gender; employment status; educational level; Fitzpatrick skin phototype; body mass index; eye and natural hair color; smoking status; leisure and occupational sun exposure; significant time spent outdoors; and mean weeks of vacation spent before the age of 10, from the ages of 11 to 18, and after the age of 18. There was a significant statistical difference among the three groups regarding history of sunburn before the age of 18 ($P < 0.001$). As expected, patients with a PMH of MM more frequently had a history of sunburn before the age of 18 than the group with AMS and the control group.

Photoprotective practices

Although 104 (86%) participants reported using sunscreen, with most of them (59/121 = 48.8%) reporting wearing sunscreen with a sun protection factor (SPF) of > 50, only 22 (18.2%) patients did so every day and only 20 (16.5%) did so all year round. Of all participants, 89 (73.6%) reported wearing sunscreen only during the summer and 94 (77.7%) only in direct sunny weather. Fifty-two patients reported reapplying sunscreen while outdoors and only a minority (37/121 = 30.58%) reported

Table 2 Sun protection practices in patients with malignant melanoma, those with atypical mole syndrome, and controls

	Patients with a PMH of MM, *n* = 26	Patients with AMS, *n* = 47	Control group, *n* = 48	All participants, *n* = 121	*P* value
Do you use sunscreen? *n* (%)					
No	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121(14)	0.461
Yes	21/26 (80 .8)	40/47 (85.1)	43/48 (89.8)	104/121 (86)	
If yes, which SPF sunblock rating do you use? *n* (%)					
< 30	2/26 (7.7)	5/47 (10.6)	9/48 (18.8)	16/121 (13.2)	0.222
≥ 30	7/26 (26.9)	10/47 (21.3)	14/48 (29.2)	31/121 (25.6)	
≥ 50	12/26 (46.2)	25/47 (53.2)	20/48 (41.7)	57/121 (47.1)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
How frequently do you use sunscreen? *n* (%)					
Everyday	5/26 (19.2)	6/47 (12.8)	11/48 (22.9)	22/121 (18.2)	0.663
Most days	4/26 (15.4)	13/47 (27.7)	10/48 (20.8)	27/121 (22.3)	
Occasionally	11/26 (42.3)	18/47 (38.3)	18/48 (37.5)	47/121 (38.8)	
Rarely	1/26 (3.8)	3/47 (6.4)	4/48 (8.3)	8/121 (6.6)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
During which seasons do you apply sunscreen? *n* (%)					
Only during the summer	17/26 (65.4)	37/47 (78.7)	30/48 (62.5)	84/121 (69.4)	0.353
All year-round	4/26 (15.4)	3/47 (6.4)	13/48 (27.1)	20/121 (16.5)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
In which of the following weather conditions do you apply sunscreen? *n* (%)					
Only in direct sunny weather	17/26 (65.4)	38/47 (80.9)	34/48 (70.8)	89/121 (73.6)	0.606
Both sunny and cloudy weather	4/26 (15.4)	2/47 (4.3)	9/48 (18.8)	15/121 (12.4)	
No sunscreen use	5/26 (19.2)	7/47 (14.9)	5/48 (10.4)	17/121 (14)	
While outdoors, do you reapply sunscreen? *n* (%)					
No	16/26 (61.5)	21/47 (44.7)	33/48 (68.8)	70/121 (57.9)	0.31
Yes	10/26 (38.5)	26/47 (55.3)	15/48 (31.3)	51/121 (42.1)	
Do you reapply sunscreen after swimming or perspiring heavily? *n* (%)					
No	14/26 (53.8)	20/47 (42.6)	28/48 (58.3)	62/121 (51.2)	0.139
Yes	12/26 (46.2)	27/47 (57.4)	20/48 (41.7)	59/121 (48.8)	
Wearing UV-protective sunglasses, *n* (%)					
Everyday	13/26 (50)	21/47 (44.7)	12/48 (25)	46/121 (38)	0.303
Most days	5/26 (19.2)	10/47 (21.3)	16/48 (33.3)	31/121 (25.6)	
Occasionally	2/26 (7.7)	7/47 (14.9)	13/48 (27.1)	22/121 (18.2)	
Rarely	1/26 (3.8)	4/47 (8.5)	4/48 (8.3)	9/121 (7.4)	
Never	5/26 (19.2)	5/47 (10.6)	3/48 (6.3)	13/121 (10.7)	
Wearing a broad-brimmed hat, *n* (%)					
Everyday	5/26 (19.2)	3/47 (6.4)	3/48 (6.3)	11/121 (9.1)	0.535
Most days	1/26 (3.8)	6/47 (12.8)	6/48 (12.5)	13/121 (10.7)	
Occasionally	6/26 (23.1)	14/47 (29.8)	13/48 (27.1)	33/121 (27.3)	
Rarely	7/26 (26.9)	13/47 (27.7)	6/48 (12.5)	26/121 (21.5)	

Never	7/26 (26.9)	11/47 (23.4)	20/48 (41.7)	38/121 (31.4)	
Wearing long-sleeved shirts or long plants made from tight fabric weave, *n* (%)					
Everyday	1/26 (3.8)	2/47 (4.3)	1/48 (2.1)	4/121 (3.3)	0.275
Most days	4/26 (15.4)	11/47 (23.4)	9/48 (18.8)	24/121 (19.8)	
Occasionally	7/26 (26.9)	13/47 (27.7)	16/48 (33.3)	36/121 (29.8)	
Rarely	5/26 (19.2)	11/47 (23.4)	12/48 (25)	28/121 (23.1)	
Never	9/26 (34.6)	10/47 (21.3)	10/48 (20.8)	29/121 (24)	
Avoiding the sun during hours of peak sunlight intensity (10:00 am to 16:00 pm), *n* (%)					
Everyday	8/26 (30.8)	9/47 (19.1)	5/48 (10.4)	22/121 (18.2)	0.492
Most days	10/26 (38.5)	20/47 (42.6)	26/48 (54.2)	56/121 (46.3)	
Occasionally	4/26 (15.4)	11/47 (23.4)	13/48 (27.1)	28/121 (23.1)	
Rarely	4/26 (15.4)	5/47 (10.6)	0/48 (0)	9/121 (7.4)	
Never	0/26 (0)	2/47 (4.3)	4/48 (8.3)	6/121 (5)	

PMH: Past medical history; MM: Malignant melanoma; AMS: Atypical mole syndrome; UV: Ultraviolet.

reapplying sunscreen after swimming or perspiring. Photoprotective practices are summarized in Table 2.

Forty-six (46/121 = 38%) patients reported daily use of UV sunglasses. There was a tendency of more frequent daily use of sunglasses in the MM and AMS groups in contrast to the control group, but this was not statistically significant (P = 0.303). Eleven (9.1%) and four (3.3%) patients reported daily use of broad-brimmed hats and long-sleeved shirts, respectively, with no significant difference among the three groups. Only a minority of patients (22/121 = 18.2%) avoided the sun daily during peak hours of sunlight intensity.

Photoprotection education and perceived barriers

Most of the patients, 90/121 (74.4%), had been given advice on how to protect their skin from sunlight, with 86/121 (71.1%) receiving that advice from their family doctor. Photoprotection education is summarized in Table 3.

One third of patients (45/121 = 37%) were given sun protection education from a health-care professional more than three times; half of them (63/121 = 52.1%) were educated from multimedia sources; and most of them (104/121 = 86%) were given written photoprotective advice.

Most of the patients (88/121 = 73%) were interested in receiving education. Eighty-eight patients (72.7%) were interested in receiving sun protection advice from a health-care worker and 74 (61.2%) were interested in receiving photoprotection advice from multimedia sources.

Half of the patients (63/121 = 52.1%) had encountered barriers that discouraged them from practicing sun protection. These barriers are summarized in Table 4. A quarter of them (27/121 = 22.3%) claimed that they did not have time to practice photoprotection measures. Concerns over adequate vitamin D levels and financial concerns were reported by 28.9% and 15.7%, respectively. Only a minority reported appearance concerns (4.1%), difficulty in obtaining materials (5.8%), or previous unpleasant experiences with and bad reactions to sunscreen (7% and 0.8%, respectively). There was no statistical difference among the three groups in our study.

DISCUSSION

To the best of our knowledge, we have here presented the first study of its kind describing demographic and clinical characteristics and assessing awareness, knowledge, attitudes, and barriers toward photoprotective practices among patients with MM and AMS and a control group. We conducted our study in the city of Heraklion, Crete, Greece, which has a very high UV index and a significantly homogeneous population. Limitations of our study include the small sample of patients and the single-center location.

Our evidence indicates that adapting effective photoprotective practices, such as the daily use of high SPF sunblock, wearing a broad-brimmed hat and a long-sleeved shirt, and avoiding sun exposure between the peak hours of 10:00 a.m. to 4:00 p.m. protect against the development of skin cancer[8-12]. Therefore, assessing photoprotective education and attitudes and providing sun protection education

Table 3 Sun protection education in patients with malignant melanoma, those with atypical mole syndrome, and controls

	Patients with a PMH of MM, *n* = 26	Patients with AMS, *n* = 47	Control group, *n* = 48	All participants, *n* = 121	*P* value
Have you ever been given advice on how to protect your skin from sunlight? *n* (%)					
No	6/26 (23.1)	9/47 (19.1)	16/48 (33.3)	31/121 (25.6)	0.59
Yes	20/26 (76.9)	38/47 (80.9)	32/48 (66.7)	90/121 (74.4)	
Have you ever received sun protection education from a family doctor? *n* (%)					
No	6/26 (23.1)	11/47 (23.4)	18/48 (37.5)	35/121 (28.9)	0.109
Yes	20/26 (76.9)	36/47 (76.6)	30/48 (62.5)	86/121 (71.1)	
On how many occasions have you received sun protection education from a healthcare professional? *n* (%)					
Never	5/26 (19.2)	10/47 (21.3)	14/48 (29.2)	29/121 (24)	0.316
Once	4/26 (15.4)	5/47 (10.6)	6/48 (12.5)	15/121 (12.5)	
Twice	3/26 (11.5)	6/47 (12.8)	8/48 (16.7)	17/121 (14)	
3 times	4/26 (15.4)	5/47 (10.6)	6/48 (12.5)	15/121 (12.5)	
3 times	10/26 (38.5)	21/47 (44.7)	14/48 (29.2)	45/121 (37)	
Have you ever received sun protection education from Media (*i.e.*, television, newspaper)? *n* (%)					
No	11/26 (42.3)	22/47 (46.8)	25/48 (52.1)	58/121 (47.9)	0.546
Yes	15/26 (57.57)	25/47 (53.2)	23/48 (47.9)	63/121 (52.1)	
Have you ever received written advice about sun protection? *n* (%)					
No	21/26 (80.8)	38/47 (80.9)	45/48 (93.8)	17/121 (14)	0.055
Yes	5/26 (19.2)	9/47 (19.1)	3/48 (6.3)	104/121 (86)	
Would you be interested in receiving education about sun protection? *n* (%)					
No	4/26 (15.4)	12/47 (25.5)	17/48 (35.4)	33/121 (27)	0.619
Yes	22/26 (84.6)	35/47 (74.5)	31/48 (64.6)	88/121 (73)	
Would you be interested in receiving photoprotection advice about sun protection from a healthcare worker? *n* (%)					
No	4/26 (15.4)	16/47 (34)	17/48 (35.4)	33/121 (27.3)	0.154
Yes	22/26 (84.6)	31/47 (66)	31/48 (64.6)	88/121 (72.7)	
Would you be interested in receiving photoprotection advice about sun protection from multimedia? *n* (%)					
No	11/26 (42.3)	16/41 (34)	20/48 (41.7)	47/121 (36.8)	0.693
Yes	15/26 (57.7)	31/41 (66)	28/48 (58.3)	74/121 (61.2)	

PMH: Past medical history; MM: Malignant melanoma; AMS: Atypical mole syndrome.

are both important and effective in preventing skin cancer, especially in areas with high UV indexes such as Crete, Greece.

Our survey highlighted that although most of the patients used sunscreen (104/121 = 86%), and half of them (57/121 = 47.1%) used sunscreen with an SPF of > 50, only a small proportion of them (22/121 = 18.2%) applied it daily, and the majority (84/121 = 69.4%) applied it only during the summer. Many participants reported never having worn a broad-brimmed hat (38/121 = 31.4%), a long-sleeved shirt, or long pants (29/121 = 24%) to protect themselves from sunlight. There was no statistical difference among the three groups regarding sun protection practices.

Most of the patients (90/121 = 74.4%) recalled having received advice on how to protect their skin from sunlight. This shows high recall of receiving photoprotective education (this number has varied

Table 4 Perceived barriers to implementation of photoprotection practices in patients with malignant melanoma, those with atypical mole syndrome, and controls

	Patients with a PMH of MM, *n* = 26	Patients with AMS, *n* = 47	Control group, *n* = 48	All participants, *n* = 121	*P* value
Have any of the following barriers discouraged you from practicing sun protection? *n* (%)					
No	7/26 (26.9%)	27/47 (57.4%)	24/48 (50%)	58/121 (47.9%)	0.656
Yes	19/26 (73.1%)	20/47 (42.6%)	24/48 (50%)	63/121 (52.1%)	
Skepticism ("I do not believe skin cancer is a serious health threat"), *n* (%)					
No	22/26 (84.6%)	43/47 (91.5%)	45/48 (93.8%)	110/121 (90.9%)	0568
Yes	4/26 (15.4%)	4/47 (8.5%)	3/48 (6.3%)	11/121 (9.1%)	
Hassle/lack of time, *n* (%)					
No	21/26 (80.8%)	35/47 (74.5%)	38/48 (79.2%)	94/121 (77.7%)	0.639
Yes	5/26 (19.2%)	12/47 (25.5%)	10/48 (20.8%)	27/121 (22.3%)	
Concerns over adequate Vitamin D, *n* (%)					
No	20/26 (76.9%)	34/47 (72.3%)	32/48 (66.7%)	86/121 (71.1%)	0.486
Yes	6/26 (23.1%)	13/47 (27.7%)	16/48 (33.3%)	35/121 (28.9%)	
Cost/financial concerns. *n* (%)					
No	25/26 (96.2%)	38/47 (80.9%)	39/48 (81.3%)	102/121 (84.3%)	0.810
Yes	1/26 (3.8%)	9/47 (19.1%)	9/48 (18.8%)	19/121 (15.7%)	
Appearance ("I do not like how using sun protection will make me look"), *n* (%)					
No	24/26 (92.3%)	44/47 (93.6%)	48/48 (100%)	116/121 (95.9%)	0.090
Yes	2/26 (7.7%)	3/47 (6.4%)	0/48 (0%)	5/121 (4.1%)	
Difficulty obtaining materials (sunscreen, sunglasses, hats, *etc*), *n* (%)					
No	25/26 (96.2%)	44/47 (93.6%)		114/121 (94.2%)	0.962
Yes	1/26 (3.8%)	3/47 (6.4%)		7/121 (5.8%)	
Sunscreen is uncomfortable or unpleasant, *n* (%)					
No	22/26 (84.6%)	43/48 (91.5%)	47/48 (97.9%)	112/121 (93%)	0.149
Yes	4/26 (15.4%)	4/48 (8.5%)	1/48 (2.1%)	9/121 (7%)	
Previous "bad" reaction to sunscreen (please specify), *n* (%)					
No	25/26 (96.2%)	47/47 (100%)	48/48 (100%)	120/121 (99.2%)	0.765
Yes	1/26 (3.8%)	0/47 (0%)	0/48 (0%)	1/121 (0.8%)	
None/no barriers have discouraged me, *n* (%)					
No	14/26 (53.8%)	21/121 (44.7%)	23/48 (47.9%)	58/121 (47.9%)	0.840
Yes	12/26 (46.2%)	26/121 (55.3%)	25/48 (52.1%	63/121 (52.1%)	

PMH: Past medical history; MM: Malignant melanoma; AMS: Atypical mole syndrome.

from 27.5% to 96% in previous papers). Our survey highlights that, despite recalling having received adequate photoprotection education, the implementation of sun protective practices in all the three groups remained suboptimal. Our study showed that adherence to photoprotective practices did not correlate with education level. Previous studies have documented that a lack of post-secondary education was correlated with a reduced adoption of sun protective behaviors[13-18].

Several barriers regarding photoprotection have been reported in the literature. In our cohort, the three most-cited barriers were "concerns over adequate vitamin D" (35/121 = 28.9%), "hassle/lack of

time" (27/121 = 22.3%), and "cost/financial concerns" (19/121 = 15.7%). Only the barrier "lack of time" was consistent with previous studies[19-23].

We also found that 72.7% of the subjects expressed interest in receiving photoprotection advice from a health-care worker and 61.2% from multimedia sources. This indicates that patients might prefer receiving verbal advice from a health-care professional, and that electronic devices might also play a crucial role in relevant education[24-27]. However, the use of multimedia methods in educating people on photoprotective practices may be inefficient for older patients.

Our study has both strengths and limitations. A dermatologist assessed all participants, and the questionnaire was not only self-reported but also the patient and the dermatologist completed the questionnaire together at the same time. The dermatologist, who examined the patient, gave more accurate data. Furthermore, the design of our study involves consecutive patients who were recruited during a specific timeline. Limitations include the small sample of patients and the single-center hospital-based nature of the study. We recruited patients and controls consecutively from a tertiary referral mole clinic who were dermatology department patients. These patients might be more motivated toward skin cancer prevention knowledge and photoprotection measures, which may limit the generalizability of our results.

CONCLUSION

Considerable efforts should be made to raise awareness regarding photoprotection practices with the aim to prevent skin cancer in patients with MM and AMS.

ARTICLE HIGHLIGHTS

Research background

Patients with atypical mole syndrome (AMS) have a 3- to 20-fold higher risk of developing malignant melanoma (MM) than individuals without.

Research motivation

The most modifiable risk factor for developing MM is the ongoing ultraviolet exposure.

Research objectives

To assess awareness, knowledge, and attitudes towards sun protection among patients with MM and AMS.

Research methods

A written survey was administered to patients with MM, those with AMS, and a control group who attended a specialist mole clinic in Heraklion in Greece.

Research results

In total 121 subjects participated in the study. Their mean age was 43.92 ± 12.55 years. There were 66 (54.4%) females and 55 (45.4%) males. Forty-seven (38.8%) patients had AMS, 26 (21.5%) had a past medical history (PMH) of MM, and 48 (39.7%) attended the clinic for a full skin checkup for their naevi without having AMS or MM. 104 (86%) participants reported using sunscreen. Approximately 74.4% of patients recalled having received advice on how to protect their skin from sunlight. The most mentioned barriers in photoprotection were concerns over adequate vitamin D and lack of time.

Research conclusions

Despite mentioning having received adequate education in photoprotection, adherence to photoprotection practices is suboptimal in patients with MM and AMS.

Research perspectives

Larger prospective studies could be performed comparing awareness, knowledge, and attitudes towards photoprotection among patients with MM and AMS before and after receiving education in photoprotection.

FOOTNOTES

Author contributions: Koumaki D, Papadakis M, and Krasagakis K contributed to designing the study; Koumaki D

contributed to collecting and analyzing the data, and writing the paper; Papadakis M contributed to analyzing the data; Kouloumvakou S contributed to collecting the data; Koumaki D, Papadakis M, and Krasagakis K contributed to revising and approving the paper.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Short term safety of coronavirus disease 2019 vaccines in patients with solid tumors receiving systemic therapy

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Abstract

BACKGROUND

There are currently three coronavirus disease 2019 (COVID-19) vaccines approved by the United States Food and Drug Administration to prevent coronavirus infection. However, robust data are unavailable on the adverse events of the vaccines in patients with solid tumor malignancies undergoing systemic therapies.

AIM

To evaluate the safety of COVID-19 vaccines in patients with solid tumors undergoing systemic therapies.

METHODS

The study included patients with solid tumors treated in an academic tertiary care center who received COVID-19 vaccination between January 1, 2021 and August 15, 2021, while undergoing systemic therapy. Electronic medical records were accessed to collect information on patient characteristics, systemic therapies, type of vaccine received, and adverse effects associated with the vaccine administration. Adverse events (AEs) were graded according to Common Terminology Criteria for Adverse Events, version 5.0.

RESULTS

The analysis included 210 patients; the median age was 70 years, and 51% of patients were female. The most common chemotherapy, immunotherapy, and targeted therapy administered were taxane-based regimens 14.2% (30/210), anti-programmed death 1 (PD-1) agents 22.8% (48/210), and antiangiogenic agents

7.1% (15/210), respectively. The most common cancers were gastrointestinal 43.8% (92/210), thoracic 30.4% (64/210), and genitourinary 17.6% (37/210). Patients received the following vaccines: 2 doses of BNT162b2 by Pfizer 52% (110/210), 2 doses of mRNA-1273 by Moderna 42% (89/210), and 1 dose of JNJ-78436735 by Johnson & Johnson 5% (11/210). At least 1 AE attributable to the vaccine was observed in 37 patients 17.6% (37/210). The total number of AEs attributable to vaccines was 62: Fifty-three grade 1 and nine grade 2. Most adverse events occurred after the second dose 59.7% (37/62). The most frequent grade 1 AEs included fatigue 17% (9/53), fever 15% (8/53), injection site reaction 13.2% (7/53), and chills 9.4% (5/53). The most frequent grade 2 AEs were fatigue 33.3% (3/9) and generalized weakness 22.2% (2/9). Therapy was delayed by 2 wk because of the AEs possibly related to vaccine administration in 3 patients 1.4% (3/210).

CONCLUSION

The present study demonstrates that the adverse events associated with COVID-19 vaccination are infrequent, mild, and rarely delay treatment in patients with solid tumors receiving systemic therapies.

Key Words: COVID-19; Adverse events; Solid tumor; Chemotherapy; Immunotherapy; Targeted therapy

Core Tip: The current study evaluates the safety and spectrum of adverse events associated with coronavirus disease 2019 (COVID-19) vaccination in solid tumor patients receiving systemic therapy. While COVID-19 vaccination has been shown to be safe and effective in the healthy population, the data confirming the safety of COVID-19 vaccines in cancer patients are sparse. The lack of safety data in cancer patients has caused significant hesitancy to receive COVID-19 vaccination among the patient population with cancer. Our study showed that the administration of COVID-19 vaccines in solid tumor patients receiving systemic therapy is safe and should be encouraged.

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INTRODUCTION

The coronavirus disease 2019 (COVID-19) pandemic, caused by infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has profoundly impacted and transformed healthcare systems across the globe. In addition to comprehensive modification in healthcare delivery, patients have encountered immeasurable emotional and socioeconomic hardships[1,2]. SARS-CoV-2 is a novel single-stranded, enveloped RNA virus that primarily spreads *via* the respiratory route and causes respiratory infection, including pneumonia with or without multiorgan failures[3]. While many patients remain asymptomatic, infection with the SARS-CoV-2 virus has been shown to cause a myriad of symptoms, including severe acute respiratory distress syndrome[1]. Analysis of comprehensive observational data has shown increased mortality, hospitalization, and intensive care admission in cancer patients who received anticancer therapy within 3 mo of infection[4,5]. A study from China reported a 3.5-fold increased risk of respiratory failure requiring mechanical ventilation in cancer patients infected with the SARS-CoV-2 virus[6]. The interplay between COVID-19 infection and cancer is complex, attributable to a wide variety of factors including immunosuppression, co-morbidities, aging, and the biology of the cancer itself[7].

The United States Food and Drug Administration approved three COVID-19 vaccines to prevent coronavirus infection. These include the BNT162b2 from Pfizer, mRNA-1273 from Moderna, and JNJ-78436735 vaccine from Johnson & Johnson. Patients with cancer should be considered a high-priority group for COVID-19 vaccination due to their higher risk of morbidity and death associated with COVID-19 disease[5,6,8-10]. However, the trials reporting efficacy and safety of COVID-19 vaccines were conducted in healthy volunteers, excluding the immunocompromised cancer patients on treatment [11-13]. Although several cancer societies recommend COVID-19 vaccination in patients with cancer, the data confirming the safety of vaccines are sparse[14,15]. This lack of rigorous scientific inquiry into vaccine safety has led to increased apprehension and hesitation to receive vaccination in the patient

population with cancer. As the incidence of cancer continues to rise, solid tumor malignancies continue to emerge among the most prevalent diagnoses. Frequently used therapeutic regimens include chemotherapy, immunotherapy, and targeted therapy. We conducted a study to assess the safety and determine the spectrum of adverse events (AEs) associated with COVID-19 vaccination in patients with solid tumors receiving systemic therapy.

MATERIALS AND METHODS

The aim of this study was to determine the real-world incidence and spectrum of AEs in patients with solid tumor malignancies receiving systemic therapy. This was a retrospective study of cancer patients who received COVID-19 vaccination between January 1, 2021 and August 15, 2021 at Froedtert and the Medical College of Wisconsin Cancer Center (Milwaukee, WI, United States of America). Inclusion criteria required that patients be at least 18 years of age at the time of inoculation and have a histologically confirmed solid tumor diagnosis for which they were receiving systemic therapy (chemotherapy, immunotherapy, or targeted therapy). Patients were excluded from the study if they had an active hematologic malignancy, were being treated with hormonal therapy, or had a benign tumor diagnosis that did not require anti-neoplastic treatment. Patients for this study were identified from the cancer center database using a tool available in the electronic medical record software (EPIC SlicerDicer tool). The initial screen identified 1480 cancer patients who received COVID-19 vaccines. Of these, 349 were omitted due to an active hematologic malignancy, and 183 patients were excluded due to diagnoses of benign solid tumors. An additional 401 patients who were receiving hormonal therapies (*i.e.*, leuprolide for prostate cancer or tamoxifen/anastrozole for breast cancer) were excluded. Finally, 337 patients were excluded who were not receiving active treatment for malignancies (*e.g.*, patients on surveillance following completion of their initial treatment) or active malignancies being treated with modalities other than chemotherapy, immunotherapy, or targeted therapies (*e.g.*, radiation therapy). After review, 210 patients were found to meet the study requirements (Figure 1). Electronic medical records for these patients were examined to collect information on patient characteristics, tumor characteristics, details of systemic therapy, type of vaccine received, and any AEs associated with the vaccine administration. Clinic and hospital notes were further analyzed to capture AEs occurring in a period between the first vaccination and day 30 after the second/final vaccination. In the case of the Johnson & Johnson vaccines, patient charts were reviewed for the 30-d period following the single dose of vaccination. AEs were graded in accordance with version 5.0 of the Common Terminology Criteria for Adverse Events [16]. The institutional review board of the Medical College of Wisconsin approved this study protocol.

RESULTS

Patient characteristic, systemic therapy, and vaccination types

Between January 1, 2021 and August 15, 2021, 210 patients were included in the study (Table 1). The median age of the cohort was 70 years (range, 23-91), 51% (108/210) of patients were female, and 87.1% (183/210) of the study population was Caucasian. Distribution of vaccine types included BNT162b2 from Pfizer 52.3% (110/210), mRNA-1273 from Moderna 42.3% (89/210), and JNJ-7843 vaccine from J&J 5% (11/210). All patients who received either the Pfizer or Moderna vaccine completed the 2-dose vaccination series. Gastrointestinal cancers were the most frequent diagnoses 43.8% (92/210), followed by thoracic cancers 30.4% (64/210) and genitourinary cancers 17.6% (37/210).

In the study cohort, 117 patients were receiving systemic chemotherapy at the time of vaccination. The median age of this cohort was 69 years, with a slight female predominance at 53% (62/117). Distribution of vaccine types were BNT162b2 from Pfizer 55.6% (65/117), mRNA-1273 from Moderna 40.1% (47/117), and JNJ-7843 vaccine from J&J 4.2% (5/117). The most common chemotherapeutic regimens included were taxane-based 25.6% (30/117) regimens followed by oxaliplatin-based regimens 22.2% (26/117).

Fifty-one patients were receiving immunotherapy at the time of vaccination. The median age of this cohort was 72 years, with a slight male predominance at 56.9% (29/51). Distribution of vaccine types were BNT162b2 from Pfizer 47% (24/51), mRNA-1273 from Moderna 45.1% (23/51), and JNJ-7843 vaccine from J&J 7.8% (4/51). The most common immunotherapeutic regimens consisted of programmed death 1 (PD-1) blocking agents 94% (48/51).

Forty-two patients were receiving targeted therapy at the time of vaccination. The median age of this cohort was 68 years, with a slight female predominance at 57% (24/42). Distribution of vaccine types were BNT162b2 from Pfizer 50% (21/42), mRNA-1273 from Moderna 45.2% (19/42), and JNJ-7843 vaccine from J&J 4.8% (2/42). The most common targeted therapy treatment administered was Osimertinib 14.2% (6/42).

Table 1 Characteristics of solid tumor patients receiving coronavirus disease 2019 vaccination

Patient characteristics	*n* = 210, %
Age at vaccination, median (range), yr	70 (23-91)
Sex	
Male	102 (49)
Female	108 (51)
Race	
Caucasian	183 (87)
African American	19 (9)
Other	8 (4)
Site of primary tumor	
Gastrointestinal	92 (44)
Thoracic	64 (30)
Genitourinary	37 (18)
Other	17 (8)
Type of systemic therapy	
Chemotherapy	117 (56)
Immunotherapy	51 (24)
Targeted therapy	42 (20)

Adverse events

The total number of AEs attributable to vaccination in the current cohort was 62 (Table 2). At least 1 unique AE was noted in 17.6% of patients (37/210). The number of patients who experienced any grade AEs was 20 in the chemotherapy group, 12 in the immunotherapy group, and 5 in the targeted therapy group. There were 33 AEs related to the Pfizer vaccine, 26 to the Moderna vaccine, and 3 to the J&J vaccine. In total, there were fifty-three grade 1 AEs 85.5% (53/62) and nine grade 2 AEs 14.5% (9/62). Following the first vaccination, there were twenty-one grade 1 and four grade 2 AEs. The most frequent grade 1 AEs were injection site reaction 23.8% (5/21), fatigue 23.8% (5/21), and fever 9.5% (2/21). The four grade 2 AEs noted included fatigue, nausea, chills, and maculopapular rash. Following the second vaccination, there were thirty-two grade 1 and five grade 2 AEs. The most frequent grade 1 AEs were fever 18.8% (6/32), fatigue 12.5% (4/32), chills 12.5% (4/32), and myalgia 12.5% (4/32). The five grade 2 AEs included 2 cases of fatigue, 2 cases of generalized muscle weakness, and 1 case of fever.

Cumulatively, the most frequent grade 1 AEs included fatigue 17% (9/53), fever 15% (8/53), injection site reaction 13.2% (7/53), and chills 9.4% (5/53). The most frequent grade 2 AEs were fatigue 33.3% (3/9) and generalized muscle weakness 22.2% (2/9). Of the grade 2 AEs, 6 were associated with the Pfizer vaccine and 3 with the Moderna vaccine. No grade 2 AEs were noted in the J&J vaccine population. In those who received the Pfizer or Moderna vaccine, the majority of AEs occurred after the second dose of vaccination 59.7% (37/62).

Treatment was delayed in 3 patients 1.4% (3/210) after the second dose of the Moderna vaccine by 2 wk because of AEs possibly related to vaccine administration. None of the patients had displayed any AEs after the first vaccination dose. Two of these 3 patients receiving immunotherapy developed generalized weakness that resolved within 2 wk without any specific treatment. The third patient developed malaise and fatigue, which also resolved spontaneously. No grade 3-5 AEs or anaphylaxis were noted in this patient cohort.

DISCUSSION

Data on the safety of COVID-19 vaccines in cancer patients undergoing systemic therapies are sparse. The current study aimed to address this unmet need by collecting data on COVID-19 vaccine-associated AEs in real-world cancer patients with solid tumors receiving various systemic therapies. The study revealed that COVID-19 vaccines cause infrequent and minor side effects in this patient population.

The pandemic caused by the novel coronavirus SARS-CoV-2 has significantly impacted cancer care delivery and cancer treatment globally. The COVID-19 pandemic has affected many aspects of cancer

Table 2 Adverse events (AEs) observed with coronavirus disease 2019 vaccination in patients with solid tumors receiving systemic therapies

	Chemotherapy	Immunotherapy	Targeted therapy
Patient number	117	51	42
Median age (yr)	69	72	68
Gender (Male/Female)	55/62	29/22	18/24
Type of vaccine administered(Moderna/Pfizer/J&J)	47/65/5	23/24/4	19/21/2
AEs (Grade 1 + 2), number (%)	37 (60)	18 (29)	7 (11)
Therapy delayed because of AEs, #	1	2	0

AEs: Adverse events.

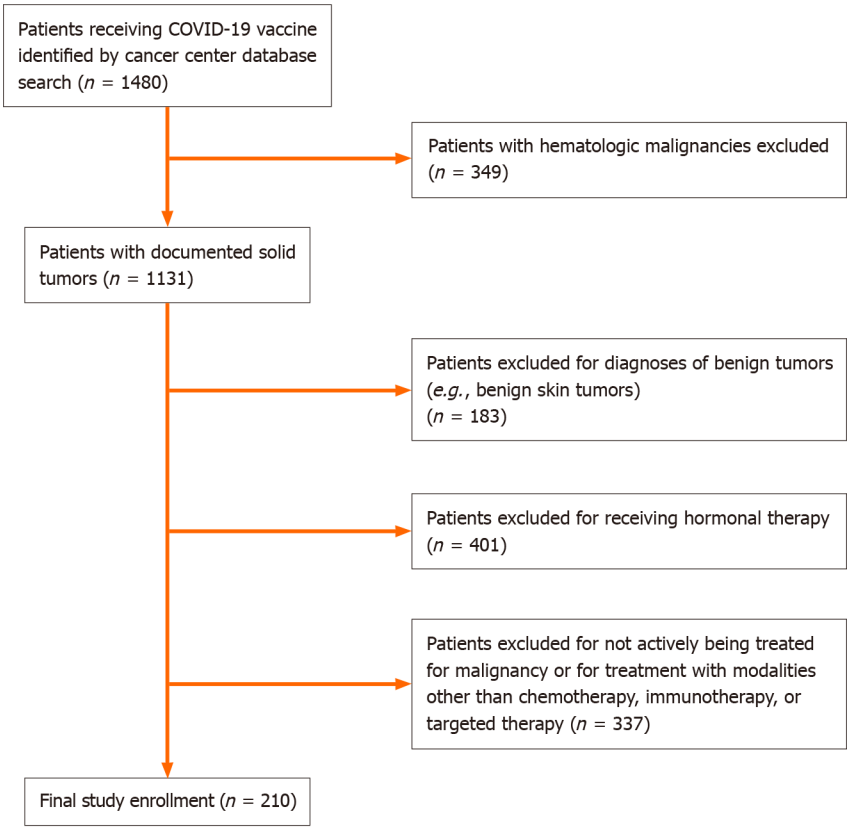

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Figure 1 Consort diagram illustrating patient enrollment. COVID-19: Coronavirus disease 2019.

care, including delay in cancer diagnosis and treatment, the long-term ramifications of which are yet to be determined[15]. The rapid development of coronavirus vaccines has brought the hope of preventing infection and restoring normalcy. While the initial clinical trials with COVID-19 vaccines demonstrated a high safety profile of the vaccines in the healthy population[11-13], limited safety data have been reported in cancer patients. Consequently, significant hesitancy in adopting widespread vaccination has been observed among patients with active cancer[17-20]. In a cross-sectional, internet-based survey, hesitancy to receive COVID-19 vaccination was reported in 13.4% of patients with cancer[19]. In a study with breast cancer patients, 26% of patients were hesitant to receive vaccination due to their concerns regarding vaccine-related AEs[20]. As patients with cancer are at increased risk of COVID-19 infection-associated complications and mortality[8-10,21,22], data confirming the safety of COVID-19 vaccines in cancer patients are urgently needed. Our study provides important safety information on COVID-19 vaccines in cancer patients undergoing active cancer treatment.

Several studies have investigated the safety of COVID-19 vaccines (summarized in Table 3). Oosting and colleagues have reported a prospective, multicenter study from the Netherlands in which patients with solid tumors received the Moderna vaccine while undergoing treatment with chemotherapy,

Table 3 Adverse events associated with coronavirus disease 2019 vaccination in published studies

Ref.	Sample size (*n*)	Cancer type	Systemic therapy	Vaccines administered	Patients with Grade 3 or worse AE, %	Immune-related AEs	Comment
Oosting *et al* [23]	544	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy	mRNA-1273 (Moderna)	10/544 (1.8%)	4% in both immunotherapy and chemoimmunotherapy group	Total 4 serious AEs were potentially related to the vaccination
Cavann *et al* [24]	257	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Chemotherapy plus biological therapy; Biologic therapy	PfizerModerna	0/257 (0%)	NA	Approximately 1/3rd of patients reported mild local reactions (pain, erythema) at the injection site
Waissengrin *et al*[25]	134	Solid Tumors	Immune checkpoint inhibitor; Chemoimmunotherapy	BNT162b2 mRNA vaccine (Pfizer)	0/134 (0%)	Nonattributable to the vaccination	Fatigue (34%), headache (16%), muscle pain (34%)
Di Noia *et al* [26]	816	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Targeted therapy	Pfizer	3.3% after the 1st dose, 1.4% after the second dose	NA	AE occurred in 359 (44%) and 301 (38.3%) patients after the first and second dose, respectively
Shmueli *et al* [27]	129	Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Biological Therapy; Hormonal Therapy; Radiotherapy	Pfizer	0/129 (0%)	NA	AE was reported by 39% of patients after the first dose and 58% of patients after the second dose- all mild to moderate in severity
Tamura *et al* [29]	120	Solid Tumor	Chemotherapy; Immunotherapy; Targeted Therapy; Chemoimmunotherapy	Pfizer Moderna	0/120 (0%); CTCAE was not used	NA	Study limited to patients receiving treatment for lung cancer only. No serious AEs or treatment delay was observed
Kian *et al*[28]	210	Solid & Non-Solid Tumors	Chemotherapy; Immunotherapy; Chemoimmunotherapy; Biological Therapy; Hormonal Therapy; Radiotherapy; Radio-hormonal; Chemo-biological	Pfizer	0.004% after 1st dose, 1.9% after the second dose	NA	AE occurred in 65 (31%) and 65 (31%) patients after the first and second dose, respectively. Injection site pain was the most common AE after both doses

AEs: Adverse events; NA: Not available.

immunotherapy, or chemoimmunotherapy[23]. In this study, the incidence of grade 3 or worse AEs were reported in 2% of patients treated with immunotherapy, 2% of patients treated with chemotherapy, and 1% of patients treated with chemoimmunotherapy. No vaccine-related death was reported. A similar study from Italy reported that patients with solid tumors undergoing active treatment also demonstrated a low incidence of significant AEs associated with COVID-19 vaccination [24]. In this study, none of the 257 evaluable patients experienced grade 3 or higher AEs. The most frequently reported AE was injection site pain and/or redness occurring in 31.5% and 33.4 % of patients after the first and second vaccinations. The most frequently reported AEs after the first dose were weakness (7%), headache (8%), and muscle pain (2.7%), and after the second dose were weakness (8.9%) and fever (5.8%). A study from Israel also reported a low incidence of AEs in patients with solid tumors receiving immunotherapy with checkpoint inhibitors, with injection site pain being the most frequently reported AE at 21% (28/134)[25]. Several other studies have demonstrated similar results[26-29]. The results of our study, in conjunction with the studies discussed above, indicate that COVID-19 vaccination is safe in solid tumor patients undergoing active treatment. The high mortality rate associated with COVID-19 disease (as high as 40% in certain patient populations)[30] and the safety data available far justify routine COVID-19 vaccination in patients with solid tumors undergoing active treatment. This recommendation is further supported by several oncology societies[14,15] and echoed by the American Society of Clinical Oncology endorsement (https://www.asco.org/covid-resources/vaccines-patients-cancer) which states: At this time, patients undergoing treatment may be offered vaccination against COVID-19 as long as any components of the vaccine are not contraindicated.

It is important to reiterate that COVID-19 vaccines in cancer patients treated with immunotherapy did not cause a higher incidence of immune-related AEs, a finding supported by several other studies [23,25]. While 2 patients in our study receiving immune checkpoint inhibitors experienced treatment delay secondary to vaccination-associated AEs, their symptoms resolved quickly with supportive care only. The remaining patients in our immunotherapy cohort demonstrated mild grade 1 AEs with rapid resolution of symptoms.

Although the current study provides valuable information on COVID-19 vaccine safety in a real-world setting, it has several limitations that include the inherent biases associated with a retrospective study design, modest sample size, and reliance on physician documentation for the data related to the AEs.

CONCLUSION

Our study demonstrates that the COVID-19 vaccines cause infrequent and mild AEs in patients with solid tumors receiving systemic therapies. The study results support routine COVID-19 vaccination in cancer patients receiving active treatment.

ARTICLE HIGHLIGHTS

Research background

In the wake of the coronavirus disease 2019 (COVID-19) pandemic, the United States Food and Drug Administration approved 3 vaccines to prevent coronavirus infection. The rapidity of vaccine approval and the limited scientific inquiry into vaccine-related adverse events notably expanded apprehension towards vaccination in patients with malignancies. Our study reports real-world data on the severity and spectrum of adverse events in solid tumor cancer patients receiving systemic therapy.

Research motivation

The motivation behind this project was to promote awareness regarding the short-term safety of COVID-19 vaccines in cancer patients with solid tumor malignancies. Our results help lessen the societal apprehension and hesitation surrounding the safety of COVID-19 vaccination.

Research objectives

The main objective of this study was to evaluate the short-term safety of COVID-19 vaccines in patients with solid tumors undergoing treatment with systemic therapies. Through rigorous analysis, we were able to document the incidence and spectrum of vaccine-related adverse events in our patient cohort. Our research forms the groundwork for future studies on long-term adverse events secondary to vaccination.

Research methods

Our study was a retrospective analysis of cancer patients who received COVID-19 vaccination between January 1, 2021 and August 15, 2021. Eligible patients were identified using the EPIC SlicerDicer tool in the Froedtert and the Medical College of Wisconsin Cancer Center database. Once identified, patients were further screened based on study inclusion/exclusion criteria. Electronic medical records for the final patients were examined to collect information on patient characteristics, tumor characteristics, details of systemic therapy, type of vaccine received, and any adverse events associated with the vaccine administration.

Research results

Analysis of our 210 patients revealed at least 1 adverse event attributable to vaccination in 17.6% of our study cohort. Of these adverse events, fifty-three were grade 1 and nine were grade 2. Our data further bolsters the sparse scientific literature regarding COVID-19 vaccination in patients with cancer.

Research conclusions

The present study demonstrates that the adverse events associated with COVID-19 vaccination are infrequent, mild, and rarely delay treatment in patients with solid tumors receiving systemic therapies. This knowledge further begs the question of whether or not patients receiving systemic therapies are mounting an appropriate response to immunogenic antigens. Further scientific inquiry exploring vaccine efficacy and adverse events in our patient cohort *vs* a healthy control group could elucidate the role of systemic therapy in vaccine-related adverse events.

Research perspectives

Future research will be focused on increasing study enrollment and exploring the long-term adverse events secondary to COVID-19 vaccination.

FOOTNOTES

Author contributions: Cox RE, Parish M, Oxencis C, McKenna E, Thapa B, and Chakrabarti S contributed equally to this work; All authors have read and approved the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Medical College of Wisconsin Institutional Review Board (Approval No. PRO00040038).

Informed consent statement: Per institutional review board approval, consent forms were not necessary since the project did not include direct contact with subjects.

Conflict-of-interest statement: Sakti Chakrabarti has received fees for serving as a speaker for Natera. Sakti Chakrabarti has received Honoraria from Haliodx and QED Therapeutics. Ronald Cox has no conflicts of interest. Marie Parish has no conflicts of interest. Carolyn Oxencis has no conflicts of interest. Bicky Thapa has no conflicts of interest. Edward McKenna has no conflicts of interest.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Decreased incidence of febrile neutropenia in Michigan following masking and social distancing orders for the COVID-19 pandemic: A population based cohort study

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Abstract

BACKGROUND

It has been theorized that 75%-80% of febrile neutropenia (FN) is caused by endogenous pathogens, while up to 20% of cases are thought to be caused by a viral infection. It is unknown if precautions such as masking and social distancing reduce the risk of FN in susceptible populations.

AIM

To determine whether coronavirus disease 2019 (COVID-19) infection mitigation efforts, namely masking and social distancing, were associated with a reduction in the incidence of FN.

METHODS

This was a retrospective population based cohort study comparing the incidence of FN in the 13 mo prior to (Year 0) and 13 mo following (Year 1) the public health executive orders (PHEO) in Michigan. Data was queried for all emergency department (ED) visits from April 1, 2019 to March 31, 2021 from the National Syndromic Surveillance Program, a program which collects data that is voluntarily submitted by approximately 89% of Michigan EDs. The primary study outcome was the incidence of FN as a proportion of ED visits in the 13-mo before and 13-mo after COVID-19 mitigations efforts, namely masking and social distancing. We hypothesized that there would be a significant decrease in the incidence of FN in the period following the PHEO aimed at reducing the spread of the severe acute respiratory syndrome coronavirus 2 virus.

RESULTS

There was a total of 8979221 total ED visits captured during the study period. In Year 0 there were 5073081 recorded ED visits and 3906140 in Year 1. There was a significant reduction in the proportion of total ED visits with a diagnosis of FN, decreasing 13.3% across periods (0.15% *vs* 0.13%, $P = 0.036$). In patients with a hematologic malignancy a more impressive reduction in the incidence of FN was evident following PHEO (22% *vs* 17%, $P = 0.02$).

CONCLUSION

We found a significant association between social distancing and mask guidelines implemented on a large public scale with decreased rates of FN, particularly in those with a hematologic malignancy. These findings may be useful in the design of future research and recommendations regarding the prevention of FN.

Key Words: Febrile neutropenia; COVID-19; SARS-CoV-2; Malignancy; Hematology; Public health

Core Tip: There has been a proven reduction in respiratory viral infections (*e.g.*, flu, common cold, *etc.*) with the implementation of social distancing and masking during coronavirus disease 2019 mitigation efforts. It has been theorized that up to 20% of febrile neutropenia is caused by viral infections. We found a significant reduction in the incidence of febrile neutropenia following the implementation of public health interventions, namely masking and social distancing, with the overall incidence of febrile neutropenia decreasing by approximately 13%. The largest reduction in febrile neutropenia was found for hematologic malignancies where the incidence of febrile neutropenia declined by 22%.

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INTRODUCTION

Febrile neutropenia (FN) is defined as neutropenia in the setting of a temperature greater than or equal to 100.4 degrees Fahrenheit and is one of the most common and costly complications associated with cancer treatment[1,2]. Granulocyte colony stimulating factor is the most effective prophylaxis against neutropenia and the progression to FN[3,4]. Yet, little is known about non-pharmacological strategies, such as masking, for the prevention of FN in at-risk populations.

Currently, FN prevention strategies include hand hygiene and the avoidance of sick contacts and crowds, however the impact of these efforts is uncertain[5]. Furthermore, the majority of FN with culture-proven bacteremia are thought to be the result of translocation of gut bacteria[6]. Although it has been theorized that 75%-80% of FN is caused by endogenous microorganisms, up to 20% of cases are thought to be caused by communicable pathogens, such as virions[7]. Accordingly, mitigating viral infection in at risks populations would theoretically reduce the incidence of FN. However, we are not aware of any population-based studies evaluating the impact of infection mitigation practices on the incidence of FN.

The public health crisis due to coronavirus disease 2019 (COVID-19) and its corresponding public health interventions provided a unique opportunity to evaluate the impact of social distancing and masking on the incidence of FN. In March of 2020, the state of Michigan issued an Executive Order that

urged residents to remain at home and socially distance, which was amended shortly thereafter to include a mask mandate[8]. The aim of our study was to evaluate the association of these mitigation efforts and the incidence of FN in patients presenting to emergency departments (EDs) in the state of Michigan.

MATERIALS AND METHODS

This is a retrospective cohort study comparing the incidence of FN in the 13 mo prior to (Year 0, March 1, 2019-March 31, 2020) and the 13 mo following (Year 1, April 1, 2020-April 31, 2021) the public health executive orders (PHEO) in Michigan. Approximately 89% of EDs across Michigan voluntarily submit encounter data (patient's chief complaint, associated diagnoses, age, sex, intake temperature, intake percent oxygen saturation, and blood pressure) to the National Syndromic Surveillance Program (NSSP). A query was made for all encounters with a diagnosis of Neutropenia (ICD-10-CM D70) from March 1, 2019-April 31, 2021. FN was defined as an intake temperature greater than or equal to 100.4 degrees Fahrenheit and an ICD-10-CM D70. The incidence of FN in Year 0 was compared to the incidence of FN in Year 1. To account for the impact of the COVID-19 pandemic on total ED visits, the incidence of each ICD code was analyzed as a proportion of ED visits for the corresponding year. Associated ICD-10-CM codes were grouped according to Clinically Relevant Groups (CRG) (Supplementary Table 1), modified from the Healthcare Cost and Utilization Project[9]. Analysis of the incidence of FN in each CRG was also performed. A visit containing multiple ICD-10-CM diagnostic codes was included in multiple CRGs, if applicable. This study was deemed exempt by the responsible institutional review board.

Statistical analysis

Descriptive statistics were generated to characterize the study cohorts. Continuous variables are described as the mean with standard deviation or median with range or interquartile range. Categorical variables are described as frequency distributions. Univariable analysis of factors associated with FN were assessed using Student's *t*-test, analysis of variance, and the chi-squared analysis. Multivariable analysis was done using logistic regression. Analyses were conducted using SPSS version 25.0 and a *P* value less than 0.05 was considered statistically significant.

RESULTS

From March 1, 2019 to April 31, 2021 there were 8979221 total ED visits in the state of Michigan with data submitted to the NSSP and at least one viable ICD-10 code. In Year 0, there were 5073081 recorded ED visits and 3906140 in Year 1, a decrease of 23% (Table 1). There was a total of 5717 encounters with a diagnosis consistent with neutropenia. There was a significant reduction in the proportion of total ED visits with a diagnosis of FN, decreasing 13.3% from Year 0 to Year 1 (0.15% *vs* 0.13%, $P = 0.036$).

In a sub-analysis of all patients with FN, in patients with a concomitant diagnosis of hematologic malignancy, FN was significantly lower in the period following PHEO (22% *vs* 17%, $P = 0.02$, Table 2). In Year 0 there was a 29.3% incidence of FN in neutropenic patients with a CRG of hematologic malignancy, *vs* a 21.2% incidence in neutropenic patients without a CRG of hematologic malignancy ($P < 0.0001$, Figure 1). This difference was not observed in Year 1 (23.8% *vs* 20.2%, $P = 0.12$). Hematologic malignancy was the only CRG diagnosis to have a relatively higher rate of FN in Year 0 compared to Year 1.

DISCUSSION

The public health response to COVID-19 in the state of Michigan provided a unique opportunity to analyze the impact of social distancing and masking on the incidence of FN. Masking and social distancing designed to prevent the spread of COVID-19 have resulted in the decline of other non-covid viral illnesses[10,11]. Our study is the first to document an association between this phenomenon and a decline in the incidence of FN, both overall and in patients with an ICD-10-CM diagnosis consistent with hematologic malignancy.

In the majority of cases, the underlying cause of FN is unknown, and therefore little is known about the efficacy of non-pharmacological efforts to prevent development of FN in neutropenic patients[12]. We found a significant association between the implementation of public health measures to prevent the spread of communicable diseases and the incidence of FN associated with hematologic malignancies. This is an important finding as patients with hematologic cancers are particularly vulnerable to FN and its associated morbidity and mortality[13]. Our findings suggest that a significant proportion of FN in patients with malignancy may have a viral etiology. Accordingly, health measures, such as masking,

Table 1 Baseline demographics

	Year 0, *n* (%)	Year 1, *n* (%)	*P* value
Total ED visits	5073081 (56.4)	3906140 (43.6)	-
Visits with neutropenia			
Male	1572 (48)	1189 (49)	0.59
Female	1704 (52)	1252 (51)	
Age (yr)	55.1 ± 23.6	54.7 ± 23.7	0.55
Visits with febrile neutropenia			
Male	2145 (48)	616 (50)	0.35
Female	2327 (52)	629 (50)	
Age (yr)	56.2 ± 22.6	50.3 ± 26.6	< 0.0001

Data reported at mean ± SD or *n* (%) unless otherwise specified. ED: Emergency department.

Table 2 Frequency of clinical relevant group diagnosis among patients with neutropenic and febrile neutropenia in Year 0 and Year 1

	Neutropenia			Febrile neutropenia		
	Year 0[1]	Year 1[2]	*P* value	Year 0[1]	Year 1[2]	*P* value
Diagnoses	*n* = 3276	*n* = 2441		*n* = 740	*n* = 505	
Common infections	1482 (45)	974 (40)	**< 0.0001**	403 (55)	255 (51)	0.17
Any malignancy	1237 (38)	833 (34)	**0.01**	276 (37)	182 (36)	0.65
Solid malignancies	722 (22)	511 (21)	0.32	120 (16)	101 (20)	0.09
Hematologic malignancies	564 (17)	357 (15)	**0.01**	165 (22)	85 (17)	**0.02**
Benign neoplasms	147 (5)	111 (5)	0.91	43 (6)	17 (3)	**0.05**
Non-malignant blood dyscrasias	1580 (48)	1213 (50)	0.27	315 (43)	227 (45)	0.41
Endocrine, nutritional, and metabolic disorders	1485 (45)	1119 (46)	0.70	268 (36)	200 (40)	0.23
Skin, musculoskeletal, psychiatric, and nervous system disorders	1224 (37)	870 (36)	0.18	211 (29)	134 (27)	0.44
Disorders of the cardiopulmonary system	1639 (50)	1125 (46)	**0.003**	324 (44)	210 (42)	0.44
Disorders of the gastrointestinal and genitourinary systems	1332 (41)	952 (39)	0.21	226 (31)	159 (32)	0.72
Coronavirus disease 2019	6 (0)	207 (9)	< 0.0001	0 (0)	45 (9)	-

[1]Includes the 13 mo prior to Michigan's state-wide public health executive orders (PHEO) and coronavirus disease 2019 (COVID-19) mitigation efforts; March 1, 2019 to March 31, 2020.
[2]Includes the 13 mo following Michigan's state-wide PHEO and COVID-19 mitigation efforts; April 1, 2020 to April 31, 2021.
Data reported at *n* (%) unless otherwise specified.

may reduce the risk of FN in vulnerable patients.

Strengths and limitation

The strengths of this study include a large number of encounters and associated accurate objective data points (ICD codes and temperature). Additionally, Michigan adopted the stay-at-home orders and mask mandates quickly and broadly, with one of the highest compliance rates of the country during Year 1 (Supplementary Figure 1)[14]. As a result, our results likely accurately reflect the effect of COVID-19 mitigation efforts on FN.

Our study has several limitations in addition to the inherent vulnerability to unmeasured biases found in retrospective studies: (1) There may be a small number of encounters of FN that are missed in this dataset; (2) Only the intake vital signs were available, and a temperature of 100.4°F was selected as the cutoff for diagnosing FN (rather than 101 F) in an effort to have a more inclusive cohort; (3) Each ED visit was treated as a separate encounter. Therefore, a patient with FN who presented to the ED on multiple occasions would be captured multiple times; (4) It is impossible to account for individual

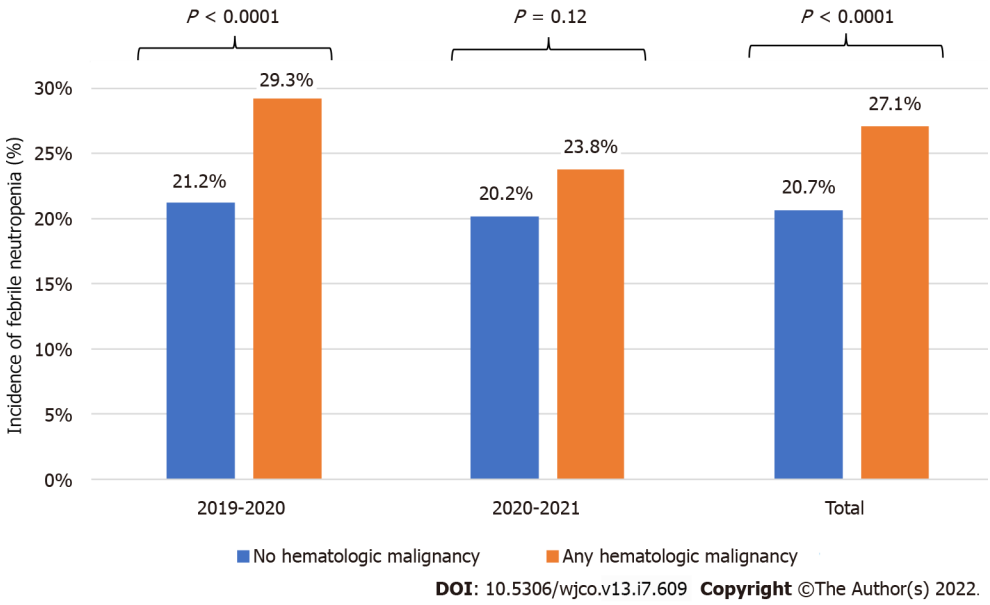

Figure 1 Incidence of febrile neutropenia in patients with an associated diagnosis of hematologic malignancy compared to those without a diagnosis of hematologic malignancy. Patients with a diagnosis of a hematologic malignancy were significantly more likely to be febrile than those without a diagnosis of hematologic malignancy in Year 0 (21.2% *vs* 29.3%, $P < 0.0001$). This difference was not seen after implementation of public health guidelines in Year 1 (20.2% *vs* 23.8%, $P = 0.12$).

patient hesitancy on presenting to ED[14]; (5) Charts review was not possible, so the effect of active myelosuppressive therapy could not be assessed; and (6) The number and identify of facilities contributing data changes over time, and the use of diagnosis codes could be inconsistent across and within facilities.

CONCLUSION

Our study found a significant association between the implementation of social distancing and mask guidelines and the incidence of FN in ED patients with neutropenia. This reduction was most pronounced in those with a hematologic malignancy. These findings may be useful in the design of clinical trials as well as informing future recommendations for the prevention of FN in vulnerable patients.

ARTICLE HIGHLIGHTS

Research background

It has been theorized that 75%-80% of febrile neutropenia (FN) is caused by endogenous pathogens, while up to 20% of cases are thought to be caused by a viral infection. It is unknown if precautions such as masking and social distancing reduce the risk of FN in susceptible populations.

Research motivation

There has been a proven reduction in respiratory viruses (*e.g.*, flu, common cold, *etc.*) with the implementation of social distancing and masking in the effort to prevent the spread of coronavirus disease 2019 (COVID-19). We sought to elucidate whether such public health measures would concomitantly reduce the incidence of FN in susceptible populations, namely those with malignancies.

Research objectives

To determine whether COVID-19 infection mitigation efforts, namely masking and social distancing, was associated with a reduction in the incidence of FN.

Research methods

This is a retrospective population based cohort study comparing the incidence of FN in the 13 mo prior to and 13 mo following the public health executive orders in Michigan. Data was queried for all emergency department visits from April 1, 2019 to March 31, 2021 from the National Syndromic

Surveillance Program.

Research results

There was a significant reduction in the proportion of total ED visits with a diagnosis of FN, decreasing 13.3% across periods (0.15% *vs* 0.13%, $P = 0.036$). In patients with a hematologic malignancy a more impressive reduction in the incidence of FN was evident following PHEO (22% *vs* 17%, $P = 0.02$).

Research conclusions

Masking and social distancing appear to decrease the risk of FN in susceptible populations, especially among patients with hematologic malignancies.

Research perspectives

Masking and social distancing appear to decrease the risk of FN in patients with malignancies, supporting the theory that a proportion of FN may be secondary to communicable infectious particles. Well-designed studies and clinical trials are needed to guide recommendations regarding masking and social distancing for the prevention of FN in vulnerable patients.

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FOOTNOTES

Author contributions: Corey L conceived of the idea; Baracy Jr MG and Corey L designed the protocol and wrote the manuscript; Baracy Jr MG and Corey L organized operative diagnoses, CPT codes, ICD-9 and ICD-10 codes and ensured the integrity of codification; Hagglund K verified the protocol was methodologically sound and analyzed the data; Arends K procured the data; Kulkarni S, Afzal F, Solomon LA, Morris RT and Aslam MF were integral in the design and execution of the project; all authors discussed the final results and contributed to the final manuscript.

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ORIGINAL ARTICLE

Retrospective Cohort Study

iCEMIGE: Integration of CEll-morphometrics, MIcrobiome, and GEne biomarker signatures for risk stratification in breast cancers

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Abstract

BACKGROUND

The development of precision medicine is essential for personalized treatment and improved clinical outcome, whereas biomarkers are critical for the success of precision therapies.

AIM

To investigate whether iCEMIGE (integration of CEll-morphometrics, MIcro - biome, and GEne biomarker signatures) improves risk stratification of breast cancer (BC) patients.

METHODS

We used our recently developed machine learning technique to identify cellular morphometric biomarkers (CMBs) from the whole histological slide images in The Cancer Genome Atlas (TCGA) breast cancer (TCGA-BRCA) cohort. Multivariate Cox regression was used to assess whether cell-morphometrics prognosis score (CMPS) and our previously reported 12-gene expression prognosis score (GEPS) and 15-microbe abundance prognosis score (MAPS) were independent prognostic factors. iCEMIGE was built upon the sparse representation learning technique. The iCEMIGE scoring model performance was measured by the area under the receiver operating characteristic curve compared to CMPS, GEPS, or MAPS alone.

Nomogram models were created to predict overall survival (OS) and progress-free survival (PFS) rates at 5- and 10-year in the TCGA-BRCA cohort.

RESULTS

We identified 39 CMBs that were used to create a CMPS system in BCs. CMPS, GEPS, and MAPS were found to be significantly independently associated with OS. We then established an iCEMIGE scoring system for risk stratification of BC patients. The iGEMIGE score has a significant prognostic value for OS and PFS independent of clinical factors (age, stage, and estrogen and progesterone receptor status) and PAM50-based molecular subtype. Importantly, the iCEMIGE score significantly increased the power to predict OS and PFS compared to CMPS, GEPS, or MAPS alone.

CONCLUSION

Our study demonstrates a novel and generic artificial intelligence framework for multimodal data integration toward improving prognosis risk stratification of BC patients, which can be extended to other types of cancer.

Key Words: Breast cancer; Gene signature; Microbiome signature; Cellular morphometrics signature; Multimodal data integration; Prognosis

Core Tip: Cancer heterogeneity consistently results in a large variation in the prognosis of patients after a certain treatment. The discovery of biomarkers for predicting prognosis can significantly assist clinical oncologists in making treatment decisions for cancer patients. Our results revealed that iCEMIGE (integration of cell-morphometrics, microbiome, and gene biomarker signatures) significantly improves risk stratification of BC patients. The clinical utility of iCEMIGE needs to be further validated in retrospective and prospective cohort studies to determine whether the iCEMIGE score can provide sufficient predictive information to stratify patients by risk and guide treatment. If so, the iCEMIGE score could assist clinicians in decision-making about cancer treatment and enable more personalized cancer therapy.

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INTRODUCTION

Cancer is a complex and heterogeneous disease that displays many morphological, genetic, and epigenetic features[1]. Cancer heterogeneity consistently results in a large variation in clinical outcomes of patients after a certain treatment[2], and therefore the development of precision medicine is essential for personalized treatment and improved clinical outcome[3-6]. The discovery of biomarkers for predicting prognosis, a critical step toward precision medicine, can significantly assist clinical oncologists in making treatment decisions for cancer patients[7-9].

Microscopic examination of the histology, which encompasses the morphological features of cancer cells, is the oldest and most basic way of cancer classification. A complete and accurate pathological cancer classification is still crucial to deciding on the best treatment plan for patients. Recently, we developed a framework powered by artificial intelligence (AI) technique for identifying cellular morphometric biomarkers (CMBs) and cellular morphometric subtypes (CMSs) from the whole slide images (WSI) of Hematoxylin and Eosin (H&E)-stained tissue histology[10,11]. We demonstrated that CMSs were significantly associated with specific molecular alterations, immune microenvironment, and prognosis in lower-grade gliomas[10].

With the rapid biotechnological development, such as next-generation sequencing, different aspects of genomic heterogeneity have been uncovered in cancers[12], which dramatically speed the discovery of molecular biomarkers for precision diagnosis and therapy. For example, several molecular biomarkers have been developed for clinical practice in breast cancer (BC)[13,14], including PAM50 (Prosigna, South San Francisco, United States), OncotypeDx (Exact Sciences Corp., Madison, United

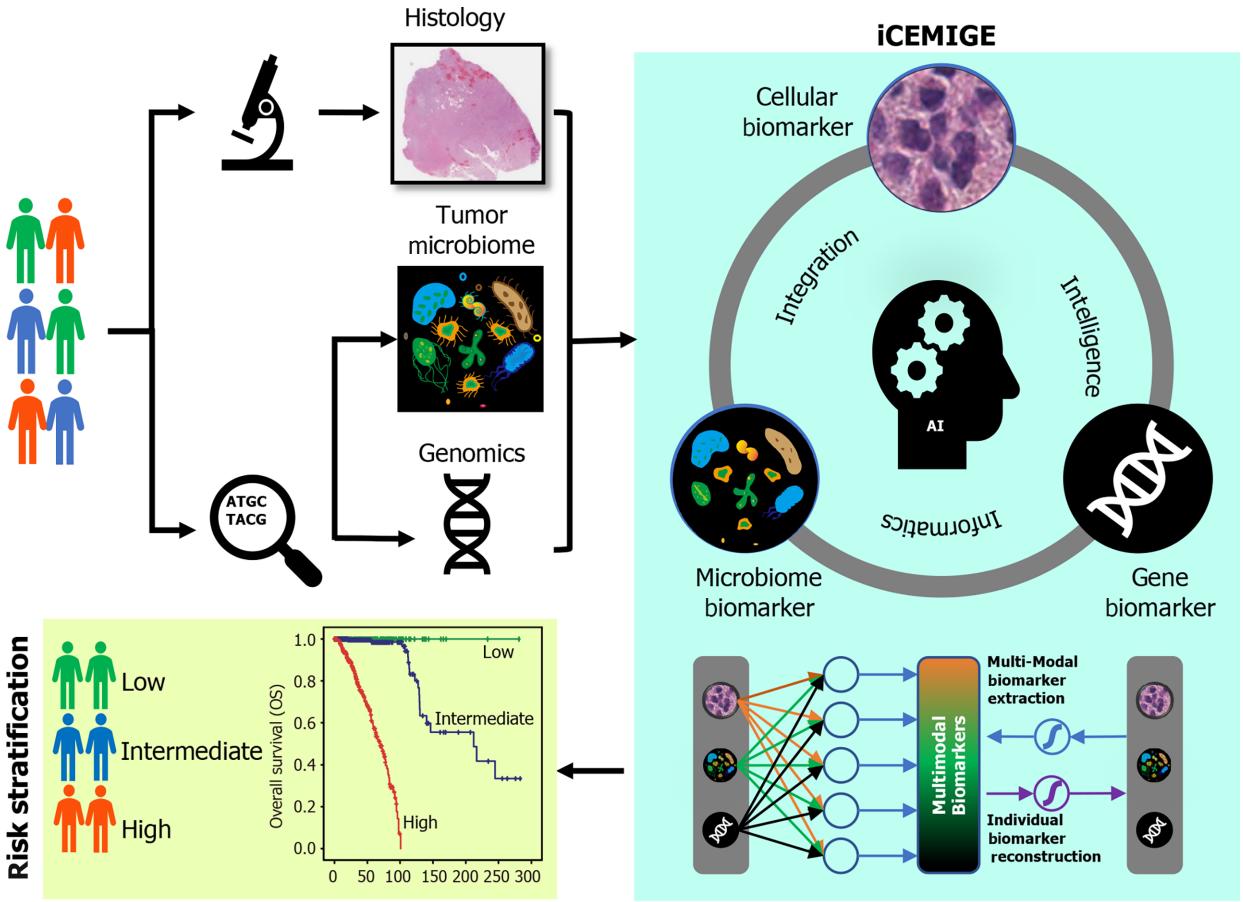

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Figure 1 A schematic illustration for the study design. Using an advanced unsupervised representation learning neural network, iCEMIGE realizes efficient and effective multi-modal biomarker mining and extraction, ensuring the optimal integration of reconstructable individual biomarkers.

States), and MammaPrint (Agendia, Amsterdam, Netherlands).

In addition to cancer genomic heterogeneity, a significant number of studies have revealed the diversity of the microbiome in cancer and the roles of the microbiome in cancer development and response to therapies[15-18]. We have recently developed a novel cancer microbiome signature for predicting the prognosis of BC patients[19]. Given the importance of tissue histology, genomics, and microbiome in cancer diagnosis and treatment, efficient and effective integration of these multimodal data is believed to open a new era for precision oncology[20].

In this study, we developed a strategy to integrate multimodal data (Figure 1) and investigated whether iCEMIGE (integration of cell-morphometrics, microbiome, and gene biomarker signatures) improves the risk stratification of BC patients. We first used our recently developed machine learning technique (CMS-ML) to identify the CMBs from the WSIs in The Cancer Genome Atlas (TCGA) breast cancer (TCGA-BRCA) cohort and established a cellular-morphometrics prognosis score (CMPS). We then demonstrated that CMPS, together with our previously reported 12-gene expression prognosis score (GEPS)[21] and the 15-microbe abundance prognosis score (MAPS)[19] were independent prognostic factors. Finally, we established the iCEMIGE scoring system and assessed its clinical value and prognosis predictive power compared to GEPS, MAPS, and CMPS alone.

MATERIALS AND METHODS

Study design and dataset

The TCGA-BRCA cohort was used in this study. The patient diagnostic tissue histology slides were downloaded from GDCportal (https://portal.gdc.cancer.gov/). TCGA-BRCA microbiome, transcriptome, and clinical data, including PAM50-based molecular subtypes, were downloaded from the cBioPortal (https://www.cbioportal.org/)[22,23]. No additional modifications were made to the downloaded data during our analyses.

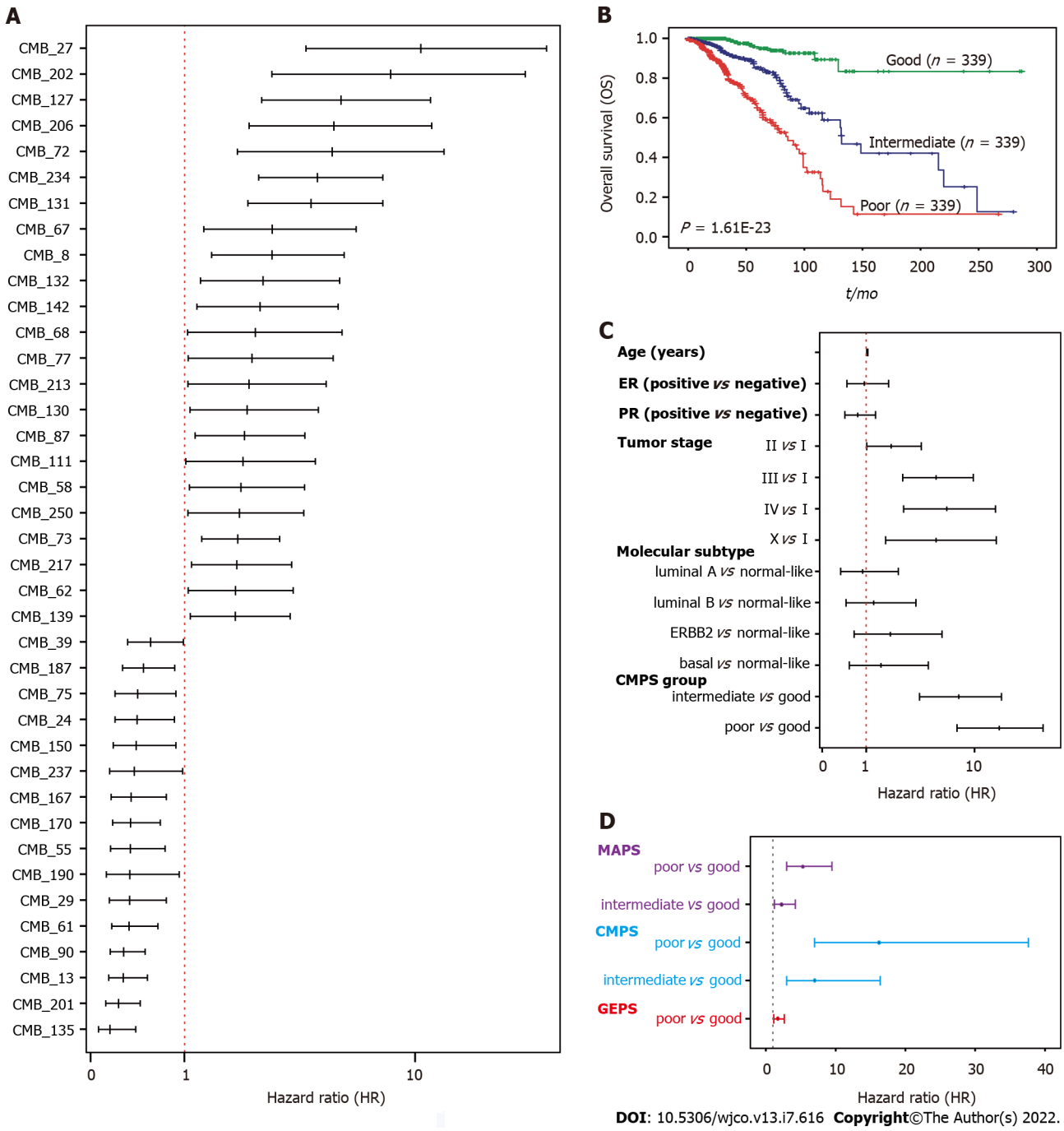

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Figure 2 Prognostic value of the cellular morphometric biomarker signature. A: Multivariate Cox regression analysis with the hazard ratio (HR) represented as a forest plot for cellular morphometric biomarkers; B: Kaplan-Meier curves on overall survival for breast cancer patients are presented with respect to the cellular morphometric prognosis score (CMPS) groups; C: Multivariate Cox regression analysis with hazard ratio (HR) represented as a forest for CMPS groups, clinical factors, and PAM50 subtypes; D: Multivariate Cox regression analysis with the HR represented as a forest plot for CMPS, MAPS, and GEPS.

Extraction of cellular morphometric characteristics and stratification of breast cancer patients

Following our previous work[10], we deployed an unsupervised feature learning pipeline, which was based on the stacked predictive sparse decomposition (SPSD)[24,25], for unsupervised discovery of underlying cellular morphometric characteristics from 15 cellular morphological features that were extracted from the diagnostic slides from the TCGA-BRCA cohort. 256 cellular morphometric biomarkers (CMB) were defined for cellular object representation. Specifically, we used a single network-layer with 256 dictionary elements (*i.e.*, CMBs) and a sparsity constraint of 30 at a fixed random sampling rate of 1000 cellular objects per WSIs from the TCGA-BRCA cohort. The pre-trained SPSD model reconstructed each cellular region (represented as a vector of 15 morphometric properties) as a sparse combination of pre-defined 256 CMBs and thereafter represents each patient as an aggregation of all delineated cellular objects belonging to the same patient.

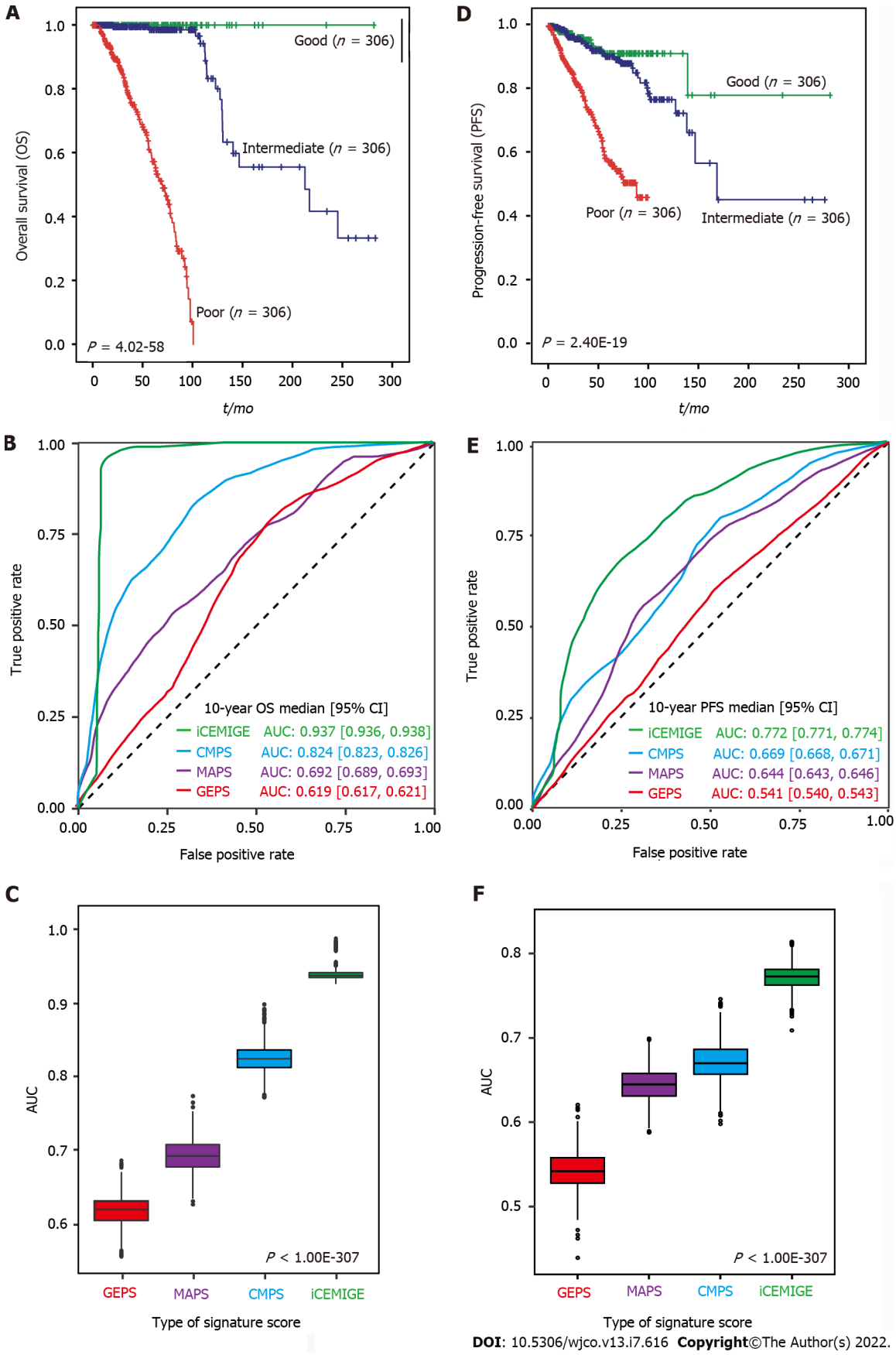

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Figure 3 iCEMIGE significantly outperforms cellular morphometric prognosis score, 15-microbe abundance prognosis score, and cellular morphometric prognosis score in prognosis prediction in the Cancer Genome Atlas breast cancer cohort. A: Kaplan-Meier overall survival (OS) curves for breast cancer (BC) patients are presented according to iCEMIGE score groups; B: ROC curves for 10-year OS prediction across different signature scores. C: Area under the curve (AUC) of 10-year OS prediction across different signature scores; D: Kaplan-Meier progress-free survival (PFS) curves for BC patients are

presented according to iCEMIGE score groups; E: Receiver operating characteristic (ROC) curves for 10-year PFS prediction across different signature scores. F: AUC of 10-year PFS prediction across different signature scores. The Kaplan-Meier p-values were calculated by the log-rank test among the three groups. The *P* values for AUC were obtained from Kruskal-Wallis test.

The prognostic effect of high or low levels of each CMB on overall survival (OS) was assessed by Kaplan-Meier analysis (survminer package in R, Version 0.4.8) and log-rank test (survival package in R, Version 3.2-3), where the TCGA-BRCA cohort was divided into two groups (*i.e.*, CMB-high and CMB-low groups) based on each CMB (survminer package in R, Version 0.4.8). The set of CMBs as a prognostic signature were selected *via* a multivariate CoxPH regression model including these CMBs with a significant effect on OS.

Finally, we calculated the cellular morphometric prognosis score (CMPS) using the formula below, where the coefficients of the final CMBs as categorical variables were obtained from multivariate CoxPH regression analysis:

$$\mathrm{CMPS} = \sum_{i=1}^{N} \left(coefficient\ of\ CMB\_Category^{i} \right) * \left(CMB\_Category^{i} \right)$$

Where N is the number of final CMBs that were independently and significantly associated with OS, and $CMB\_Category^{i}$ is the category of the i^{th} CMB (*i.e.*, CMB-high: 1; CMB-low: 0).

Mining of multi-modal iCEMIGE biomarker signature

We extended the unsupervised feature learning pipeline (SPSD)[24,25] to achieve efficient and effective mining of multi-modal biomarker signatures from prebuilt cellular-morphometrics, microbiome, and gene biomarkers. Given $X = [x_1,\ldots,x_N] \in R^{m\times N}$ as a set of patients (N) with a combination of biomarkers from different modalities (*i.e.*, cellular-morphometrics, microbiome, and gene biomarkers), the formulation of the iCEMIGE multi-modal biomarker mining model was defined as follows.

$$\boldsymbol{min}_{\mathbf{B,Z,W,G}} \ /\mathbf{X} - \mathbf{BZ}/_F^2 + /\mathbf{Z} - \mathbf{G}\sigma(\mathbf{WX})/_F^2 + \lambda_1 /\mathbf{Z}/_1$$

$$\boldsymbol{s.t.}\ /\boldsymbol{b_i}/_,^2 = \mathbf{1}, \forall \boldsymbol{i} = \mathbf{1}, \ldots, \boldsymbol{h}$$

Where $B = [b_1,\ldots,b_h] \in R^{m\times h}$ was a set of multi-modal biomarkers to be mined. Each multi-modal biomarker (b) was composed of *m* individual biomarker (*e.g.*, m = 66 in our study); $Z = [z_1,\ldots,z_N] \in R^{h\times N}$ was the sparse multi-modal biomarker expression matrix, where z_i was the sparse multi-modal biomarker expression profile of the original patient biomarkers (x_i), consisting of relative abundances of all (*h*) multi-modal biomarkers that contributed to the reconstruction of x_i; $W \in R^{h\times m}$ was the auto-encoder for efficient and effective extraction of sparse multi-modal biomarker expression matrix (*Z*) from original patient biomarker data (X); $G = diag\ (g_1,..,g_h) \in R^{h\times h}$ was a scaling matrix with *diag* being an operator aligning vector $[g_1,..,g_h]$, along the diagonal; $\sigma(\cdot)$ was an element-wise sigmoid function; λ_1 was the regularization constant to ensure the sparsity of *Z*, such that only a subset of multi-modal biomarkers was utilized during the reconstruction of original patient biomarker data.

The first constraint: $\| \mathbf{X} - \mathbf{BZ} \|_{\mathbf{F}}^{\mathbf{2}}$, penalized the reconstruction error of original patient biomarker data (X) with multi-modal biomarker (B) and the corresponding sparse multi-modal biomarker expression matrix (*Z*), which helped minimize the loss of individual biomarker information; the second constraint: $\| \mathbf{Z} - \mathbf{G}\sigma(\mathbf{WX}) \|_{\mathbf{F}}^{\mathbf{2}}$, penalized the approximation error of sparse multi-modal biomarker expression matrix (*Z*) with the auto-encoder, which helped improve the accuracy of multi-modal biomarker extraction for new patients; the third constraint: $\| Z \|_1$, penalized the sparsity of the multi-modal biomarker expression matrix, which helped ensure the utilization/activation of dominant multi-modal biomarkers during the learning process.

Construction of the iCEMIGE score

After multi-modal biomarker mining (*i.e.*, 256 multi-modal biomarkers mined in this study), a multivariate Cox regression was performed on 256 multi-modal biomarker signatures, defined as 256 covariates using the TCGA-BRCA dataset. The iCEMIGE score of each patient was calculated by the following formula:

$$iCEMIGE\ score = \sum_{i=1}^{256} (\text{ covariate } i\ coefficient) * (\text{ covariate } i\ expression\ level)$$

Nomogram, receiver operating characteristic and C-index

A nomogram model (rms package in R, Version 6.0-1) was constructed to predict 5- and 10-year OS probability of BC patients. The time-dependent receiver operating characteristic (ROC) curve (survival ROC package in R, Version 1.0.3) and concordance index (C-index) were used to evaluate the

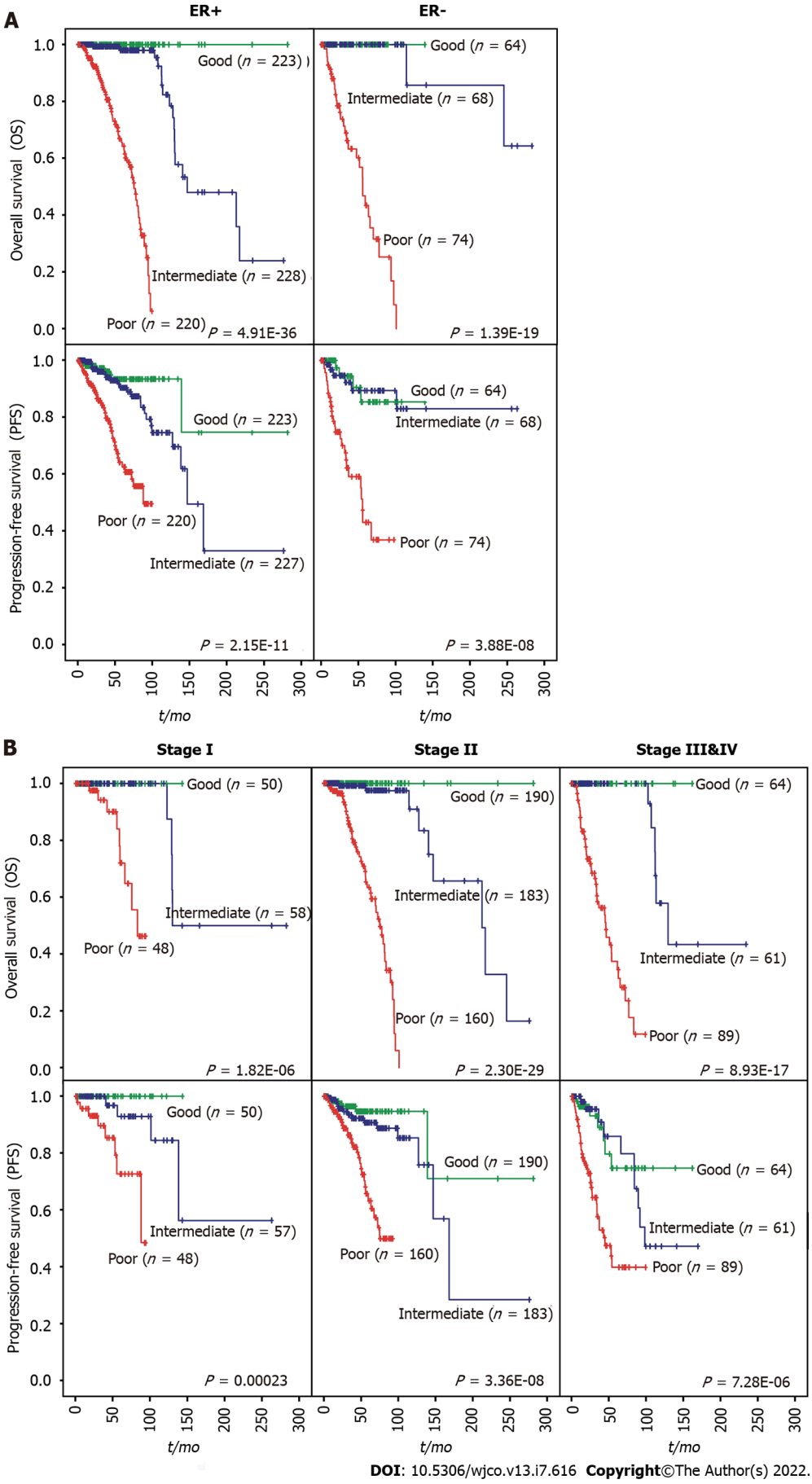

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Figure 4 Prognostic value of iCEMIGE score on overall survival and progress-free survival according to ER status and tumor stage. A:

Kaplan-Meier curves on overall survival (OS) (top panel) and progress-free survival (PFS) (bottom panel) for ER+ and ER- breast cancer (BC) patients are presented according to iCEMIGE score groups; B: Kaplan-Meier curves on OS (top panel) and PFS (bottom panel) for Stage I, II, and III&IV BC patients are presented according to iCEMIGE score groups. The *P* values were obtained from the log-rank test among the three groups.

performance of the nomogram model, where the C-index was repeated with 1000 bootstrapping iterations and an 80% sampling rate per iteration. Mann-Whitney non-parametric test was used for the comparison across models.

Statistical analysis

The cohort of patients were divided into three groups (Poor: top third; Intermediate: middle third; and Good: bottom third) based on CMPS or iCEMIGE score. The independent prognostic impact of different scores (CMPS and iCEMIGE) was assessed by multivariate CoxPH regression including the clinical factors (age, stage, ER, and PR status) and PAM50-based molecular subtype. All statistical analyses were performed through either SPSS 24.0 (IBM, NY, United States) or R (version 4.0.2, https://www.r-project.org/). Graphic visualizations were generated by R (ggpubr package, Version 0.4.0; ggplot2 package, Version 3.3.3) or SPSS. The statistical significance was defined as $p<0.05$ (two-tails).

RESULTS

Identifying cellular morphometric biomarkers for prognosis of BC patients

Over 300 million cellular objects from 1085 diagnostic slides of 1017 TCGA-BRCA patients were recognized and delineated by an unsupervised feature learning pipeline based on SPSD[24]. Each cellular object was represented with 15 morphometric properties as described in our previous work[10].

Next, we optimized and trained our SPSD model based on pre-quantified cellular objects randomly selected from the TCGA-BRCA cohort to discover the underlying cellular morphometric biomarkers (CMBs). After training, the prebuilt SPSD model reconstructed each cellular object as a sparse combination of the pre-identified 256 cellular morphometric biomarkers, which led to the novel representation of every single cellular object as 256 sparse code (reconstruction coefficient); and thereafter, the corresponding 256-dimensional cellular morphometric context representation of each patient as an aggregation of all delineated cellular objects belonging to the same patient (Supplementary Table 1). The final patient-level cellular morphometric context representation consisted of 256 CMBs.

We next evaluated the association of 256 CMBs with OS in the TCGA-BRCA cohort. Survival analysis revealed that 148 of 256 CMBs had a significant prognostic impact ($p < 0.05$, Supplementary Table 2). Among these 148 CMBs, 39 CMBs demonstrated independent and significant association with OS by multivariate CoxPH regression analysis (Figure 2A; Supplementary Figure 1; Supplementary Table 3), which were defined as a 39-CMB signature.

Assessing prognostic value of the 39-CMB signature

To further evaluate the prognostic value of the 39-CMB signature, we constructed the cellular morphometric prognosis score (CMPS) (see Methods) and divided TCGA-BRCA cohort into three groups (Poor: top third; Intermediate: middle third; and Good: bottom third) based on CMPS (Supplementary Table 4). Patients with good scores had significantly longer OS than those with poor scores. The OS of patients with intermediate scores was between these two groups (P = 1.61E-23, Figure 2B). Moreover, CMPS provided additional prognostic value to clinical factors (age, ER, PR, and stage) and PAM50-based molecular subtypes (Figure 2C).

Establishing the iCEMIGE prognostic model

Omics analyses of cancers have further revealed their genomic heterogeneity. FDA has approved many genomic biomarkers for clinical use, such as PAM50. Based on the omics data, we have previously identified 12-gene[21] and 15-microbe signatures[19] for the prognosis of BC patients (Supplementary Table 3). We conducted a multivariate Cox regression analysis to address whether GMPS, MAPS, and GEPS are independent prognostic factors. Indeed, CMPS, MAPS, and GEPS were significantly and independently associated with OS (Figure 2D). We then integrated 39 CMBs, 15 microbes, and 12 genes in an unsupervised representation framework ("iCEMIGE") and mined 256 multi-modal biomarkers (Supplementary Table 3) with experimentally optimized parameters for C-index for OS (Supplementary Figure 3). The optimal iCEMIGE score was then constructed to assess a patient's risk for death and disease progression (Supplementary Table 4, details see Materials and Methods).

Evaluating the prognostic value of the iCEMIGE score

A total of 919 BC patients in the TCGA-BRCA cohort with full signature (iCEMIGE) data were included in this evaluation (Supplementary Table 5). 919 BC patients were stratified into different prognostic

groups (Poor: top third; Intermediate: middle third; and Good: bottom third) according to the iCEMIGE score. Patients within the poor prognosis group had significantly shorter OS compared to those within the intermediate and good prognosis groups (P = 4.02E-58, Figure 3A). Importantly, we showed that the iCEMIGE score was more effective in predicting OS of BC patients than CMPS, MAPS, and GEPS alone (Figure 3B and C; Supplementary Figure 2A and B). Moreover, we found that the iCEMIGE score was also significantly associated with PFS (P = 2.40E-19, Figure 3D) and had more effective in predicting PFS (Figure 3E and F; Supplementary Figure 2C and D).

We then evaluated whether the prognostic value of the iCEMIGE score was independent of ER status, stage, and molecular subtypes. As shown in Figure 4A, patients with poor iCEMIGE scores had significantly shorter OS and PFS compared to those with good iCEMIGE scores in both ER+ and ER- groups. Moreover, the iCEMIGE score was significantly associated with OS and PFS in all different stages (Figure 4B) and subtypes (Figure 5).

Finally, using multivariate Cox regression analyses (including pathological stage, age, PR status, ER status, molecular subtype, iCEMIGE), we demonstrated that iCEMIGE was an independent prognostic factor for both OS (Figure 6A) and PFS (Supplementary Figure 4A). These findings indicate that the iCEMIGE score has an independent prognostic value in BCs.

To further assess the clinical value of the iCEMIGE score, we established a nomogram model, a valuable clinical tool for prognosis prediction, where we integrated iCEMIGE with clinical factors (age, stage, ER, and PR), PAM50-based molecular subtypes to predict the 5- and 10-year OS probability of BC patient (Figure 6B). The iCEMIGE score significantly improved the predictive power of prognosis (Figure 6C). Similar results were found for PFS (Supplementary Figure 4B and C).

DISCUSSION

High BC heterogeneity brings up a significant challenge for predicting a patient's response to treatment or prognosis. In this study, we established a new strategy for tackling this challenge by integrating multimodal signatures and demonstrated that such approach significantly improved the power for prognostic prediction compared to the single modal biomarker. In addition, we showed that iCEMIGE is significantly superior in predicting OS and PFS compared to the PAM50-based molecular subtype in the TCGA-BRCA cohort, although additional validation is required, as stated later in the limitations of this study.

The majority of biomarker developments are limited to a single modal data[20]. In the past, we followed the same path to define the 12-gene expression prognosis score (GEPS)[21] and the 15-microbe abundance prognosis score (MAPS)[19] in BC. Here, we developed the 39-CMB prognosis score (CMPS) using an AI-driven CMB detection technique[10]. We found that CMPS, MAPS, and GEPS had an independent prognostic value. This suggests that different modal data provide unique clinical value for prognosis prediction and raises the possibility that integrating multimodal biomarkers can advance precision oncology by more accurately predicting the risk of treatment failure, relapse *etc.*

Integrating multimodal data to yield improved performance compared with each modality alone remains challenging. In this study, we presented a multi-step approach to integrate cellular morphometric, molecular, and microbiome landscapes into a multimodal prognostic system for BC. Firstly, we identified the biomarker signature and systematically assessed its prognostic value in each type of modal data. Secondly, we investigated whether these modal-specific biomarker signatures are independent prognostic factors. Thirdly, we established the final predictive model incorporating all modal biomarker signatures with significantly improved prognostic risk stratification compared with each modality alone. Finally, we systematically evaluated the clinical value of the final predictive model. Such a strategy can extend to other types of cancers.

Modern clinical instruments are generating massive amounts of multimodal data, including radiology, histology, and molecular data, where each of them provides unique value for cancer diagnosis and treatment. Therefore, the efficient and effective integration of multimodal data becomes critical and, however, remains challenging in terms of robustness, interpretability, and translational impact, even with the current advancesin artificial intelligence techniques[26-28]. Two major trends in multimodal integration in cancer research are modal-specific raw data integration (MDI)[29,30] and modal-specific representation integration (MRI)[31,32]. The MDI strategy handles each modality (*e.g.*, histology and genomics) using different neural network structures and then combines the corresponding output of each neural network branch in subsequent network layers to predict the health outcome. Trained in an end-to-end fashion (*i.e.*, black-box fashion), this strategy delivers a convenient and powerful utilization of information and interaction across modalities; however, in general, it lacks biomedical interpretability. In addition, such a strategy does not guarantee the learning of clinically significant and independent information per each modality, and thus the alternative deployment of an individual modality or a subset of modalities is nearly impossible.

In contrast, the MRI provides a stepwise strategy, where the first step consists of outcome-driven representation mining per modality, and the second step integrates modal-specific representation towards the outcome. Obviously, MRI is more likely (without guarantee) to mine model-specific repres-

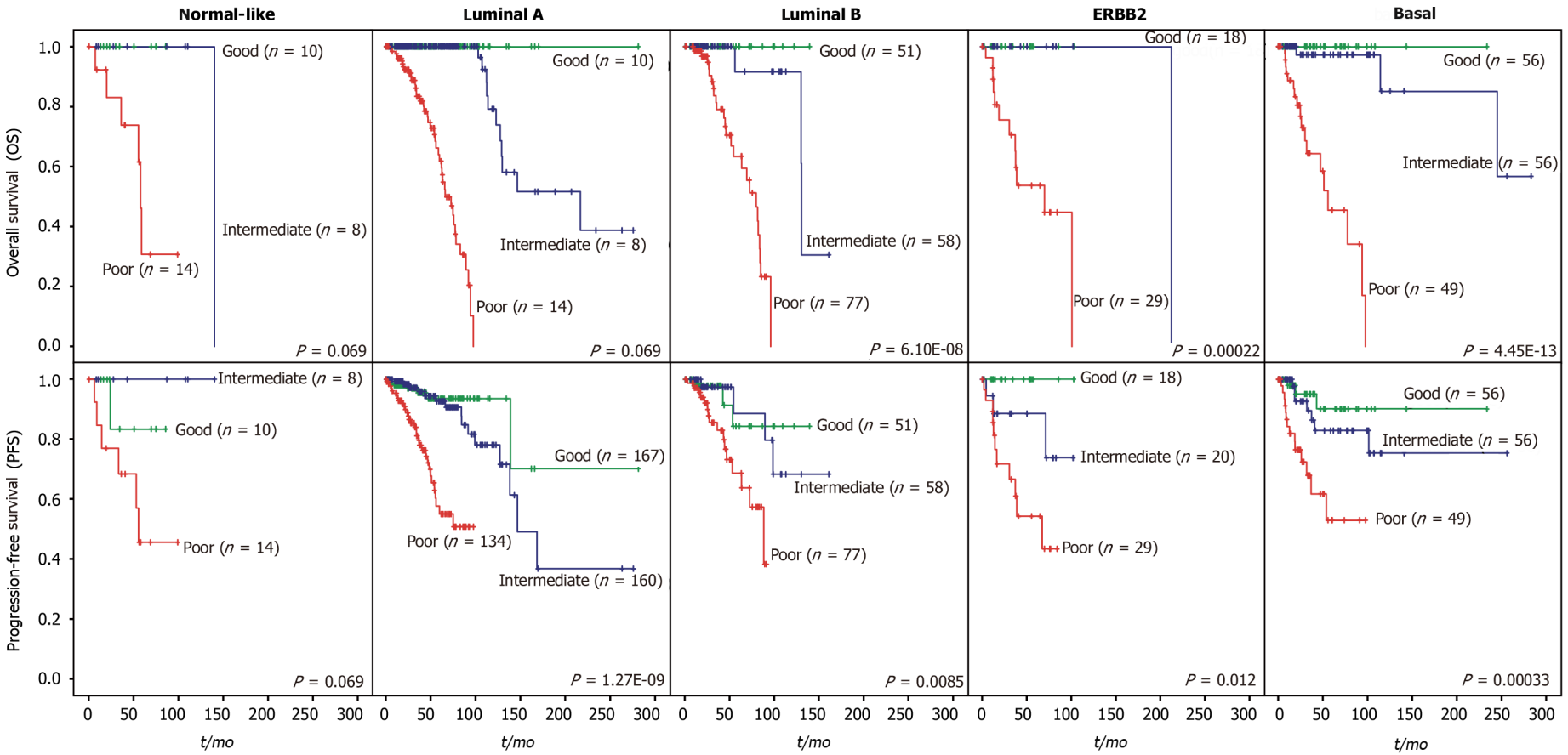

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Figure 5 Prognostic value of iCEMIGE scores on overall survival and progress-free survival within different molecular subtypes. Kaplan-Meier curves on overall survival (top panel) and progress-free survival (bottom panel) for breast cancer patients are presented with respect to the iCEMIGE score groups in different molecular subtypes. The *P* values were calculated by the log-rank test among the three groups.

entation with independent clinical value *via* a stepwise mechanism and consequently provides more flexibility in individual/subset modality deployment. This flexibility is important in clinical practice, especially when all modalities are not available. Extended from the MRI strategy, our work realizes the modal-specific knowledge integration (MKI) by enforcing the mining and utilization of biomedically interpretable, clinically significant and independent, and double-blindly validated knowledge (*i.e.*, cellular morphometric biomarkers, microbiome biomarkers, and genomic biomarkers) through an AI-powered systems biology workflow for maximized clinical implications and translation impact.

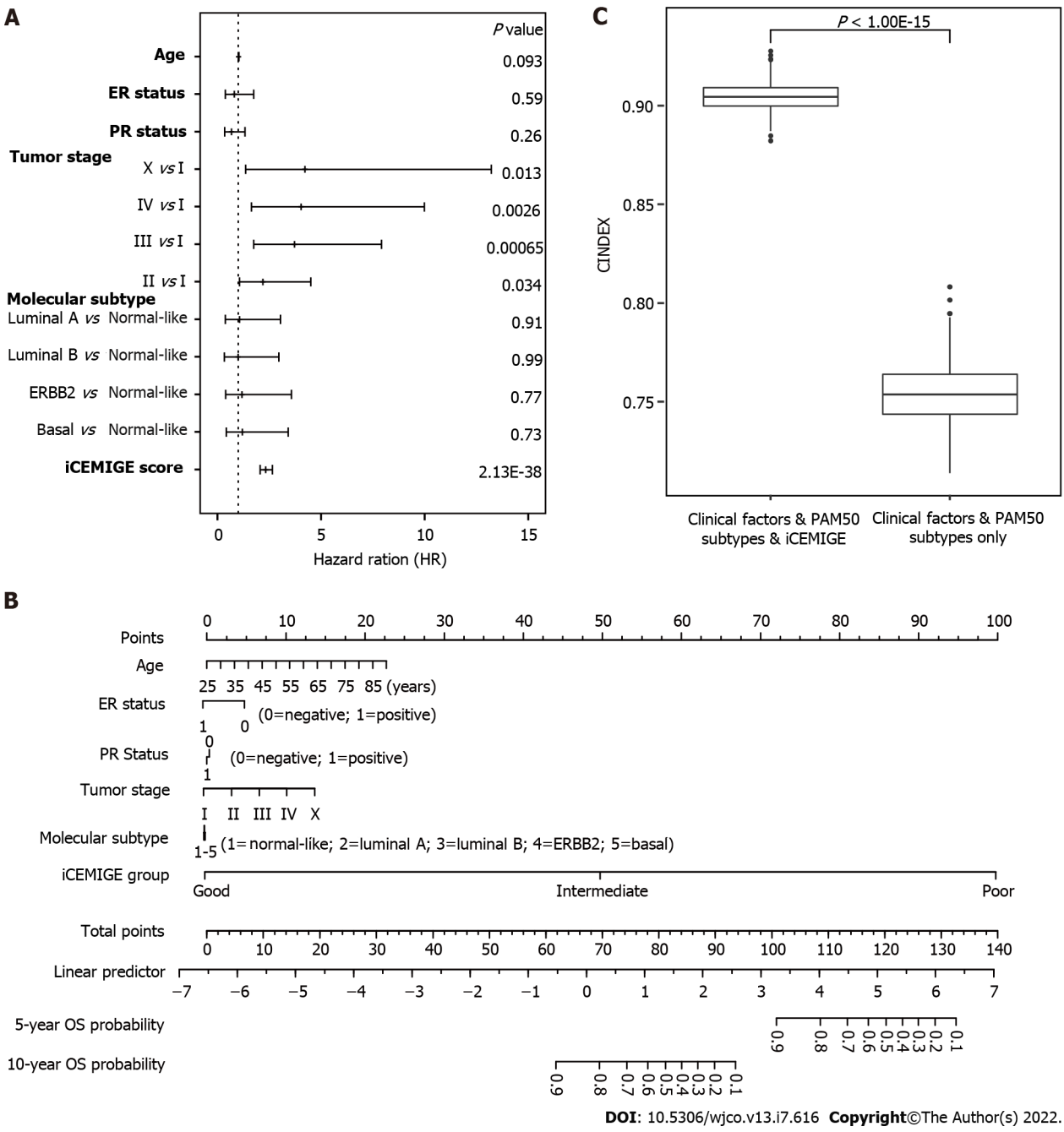

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Figure 6 iCEMIGE score provides significant and additional value for overall survival prediction. A: Multivariate Cox regression analysis of overall survival (OS) with hazard ratio represented as a forest for iCEMIGE score, clinical factors, and PAM50 subtypes; B: Nomogram for predicting OS was constructed based on integrating clinical factors and molecular subtype with iCEMIGE; C: C-index comparison for OS in different nomogram models with and without iCEMIGE. The *P* value was calculated by Mann-Whitney non-parametric test.

Our study established a new promising strategy for integrating multimodal data to enhance prognostic prediction. A significant limitation was that we did not have independent cohorts to validate our findings. In addition, due to the limited clinical information in the TCGA-BRCA cohort, we were unable to comprehensively explore the potential confounding clinical factors, including tumor size, different cancer treatments, *etc*. The clinical utility of iCEMIGE needs to be further validated in retrospective and prospective cohort studies to determine whether the iCEMIGE score can provide sufficient predictive information to stratify patients by risk and guide treatment. If so, the iCEMIGE score could assist clinicians in decision-making about cancer treatment and enable more personalized cancer therapy.

CONCLUSION

Our study demonstrates a novel and generic AI framework for multimodal data integration toward improving prognosis risk stratification of BC patients, which can be extended to other types of cancer.

ARTICLE HIGHLIGHTS

Research objectives

To develop a strategy to integrate multimodal data and to investigate whether iCEMIGE (integration of cell-morphometrics, microbiome, and gene biomarker signatures) improves the risk stratification of breast cancer patients.

Research motivation

Modern clinical instruments are generating massive amounts of multimodal data, including radiology, histology, and molecular data, where each of them provides unique value for cancer diagnosis and treatment. Efficient and effective integration of these multimodal data is believed to open a new era for precision oncology.

Research background

Cancer heterogeneity consistently results in a large variation in clinical outcomes of patients after treatment. The discovery of biomarkers for tailoring cancer treatments is a critical step toward personalized medicine.

Research perspectives

The iCEMIGE score could assist clinicians in decision-making about cancer treatment and enable more personalized cancer therapy.

Research conclusions

Our study indicates that multimodal integration (iCEMIGE) can more accurately predict the prognostic risk of breast cancer patients.

Research results

iCEMIGE is significantly superior in predicting overall and progression-free survival of breast cancer patients compared to single modal biomarker and the PAM50-based molecular subtype, which is one of FDA approved biomarkers and is currently used in clinical practice.

Research methods

The artificial intelligence pipeline powered is used to identify cellular morphometric biomarkers. Single modal biomarker signatures are integrated using the sparse representation learning technique to establish iCEMIGE. Clinical value of iCEMIGE is evaluated using different statistical methods.

FOOTNOTES

Author contributions: Perez-Losada J, Chang H, and Mao JH planned the project; Chang H, Mao XY, Perez-Losada JP, and Mao JH wrote the manuscript; Mao XY, Chang H, and Mao JH designed the algorithm, performed the bioinformatics analyses, and conducted statistical tests; Abad M, Rodríguez-González M, and Rodríguez CA provided pathological and clinical interpretation; All authors have read and edited the manuscript; Chang H and Mao JH are accountable for communications with requests for reagents and resources; Mao JH and Chang H contributed equally to these senior authors.

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Informed consent statement: The data used in this study are from the public databases. Therefore, the informed

consent is not applicable.

Conflict-of-interest statement: All the authors declare no conflicts of interest.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Clinical characteristics and outcomes in carbohydrate antigen 19-9 negative pancreatic cancer

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Abstract

BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of death from cancer worldwide. Tumor markers like carbohydrate antigen 19-9 (CA 19-9) have been proven valuable as a diagnostic tool and a predictor for tumor staging and response to therapy.

AIM

To delineate the phenotype of normal CA 19-9 PDAC according to clinical features, disease staging and prognosis as compared with high CA 19-9 PDAC cases.

METHODS

We performed a retrospective single-center analysis of all PDAC cases admitted in our Gastroenterology department over a period of 30 mo that were diagnosed by endoscopic ultrasound-guided tissue acquisition. Patients were divided into two groups according to CA 19-9 levels over a threshold of 37 U/mL. We performed a comparison between the two groups with regard to demographic and clinical

data, biomarkers, tumor staging and 6-mo survival.

RESULTS

Altogether 111 patients were recruited with 29 having documented normal CA 19-9 (< 37 U/mL). In the CA 19-9 negative group of patients, 20.68% had elevated levels of both CEA and CA 125, 13.79% for CA 125 only whilst 17.24% for CEA only. The two groups had similar demographic characteristics. Abdominal pain was more frequently reported in positive *vs* negative CA 19-9 PDAC cases (76.83% *vs* 55.17%), while smoking was slightly more prevalent in the latter group (28.04% *vs* 31.03%). Tumors over 2 cm were more frequently seen in the positive CA 19-9 group, reflecting a higher proportion of locally advanced and metastatic neoplasia (87.7% *vs* 79.3%). Six-month survival was higher for the negative CA 19-9 group (58.62% *vs* 47.56%).

CONCLUSION

Elevated CA 19-9 at diagnosis seems to be associated with a more pronounced symptomatology, high tumor burden and poor prognosis compared to negative CA 19-9 PDAC cases. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

Key Words: Pancreatic cancer; Carbohydrate antigen 19-9; Survival; Lewis; Outcome

Core Tip: Given the large heterogeneity of pancreatic cancer, delineation of subgroups with different tumor biology is essential for personalized management. We outlined the phenotype of carbohydrate antigen 19-9 negative pancreatic cancer according to clinical features, disease staging and prognosis.

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDAC) is a leading cause of death from cancer worldwide, mostly due to late-stage diagnosis and resistance to chemotherapy. According to Globocan statistics 2020, pancreatic cancer has an incidence rate of 4.9/100000 and mortality almost equal to its incidence of 4.5/100000[1]. In fact, while mortality rates from other types of cancer are decreasing, pancreatic cancer is the only malignancy with an unfavorable trend[2].

Because of its aggressive tumor biology, early diagnosis is very important in order to maximize outcomes. Several strategies have been considered for setting an early accurate diagnosis, from case-finding tools to surveillance of high-risk patients. Alongside the imaging evaluation, there is a great interest in the development of biomarkers for optimizing the management of pancreatic adenocarcinoma[3].

The most commonly used biomarker for PDAC is carbohydrate antigen 19-9 (CA 19-9), which is related to Lewis blood group antigens, and has been proven valuable as a diagnostic tool and in tumor staging, resectability and response to therapy[3]. CA 19-9, also called sialylated Lewis (a) antigen, is synthesized by pancreatic and biliary ductal cells and by other types of epithelium (stomach, colon, uterus, lung, salivary glands), which makes it a nonspecific biomarker for PDAC[4,5]. Elevated CA 19-9 has been reported in both benign and malignant pathology (Figure 1)[6,7]. Expression of CA 19-9 requires the presence of Lewis antigens A [Le(a+b-)] or B [Le(a-b+)], meaning that [Le(a-b-)] are theoretically non-producers of CA 19-9[8]. Lewis negative individuals ([Le(a-b-)]) lack the enzyme α1-3,4 fucosyltransferase, which is required for CA 19-9 biosynthesis. This dysfunction of the Lewis gene is associated with deficient protein fucosylation, which has been involved in cancer development[9].

As CA 19-9 secretion is dependent on the Lewis antigen expression, undetectable false negative results can occur in Lewis antigen-negative individuals, meaning [Le(a−b−)] non-expressors[10]. This could represent a cause of delayed diagnosis in these patients and a pitfall in screening strategies based on CA 19-9. While red cell phenotyping for Lewis antigen status would provide insight in such situations, this is not routinely performed in clinical practice. However, despite the relationship between CA 19-9 secretion and Lewis antigen status, not all Lewis negative individuals with PDAC are non-secretors of CA 19-9, which makes CA 19-9 retain its diagnostic utility at least partially even in this

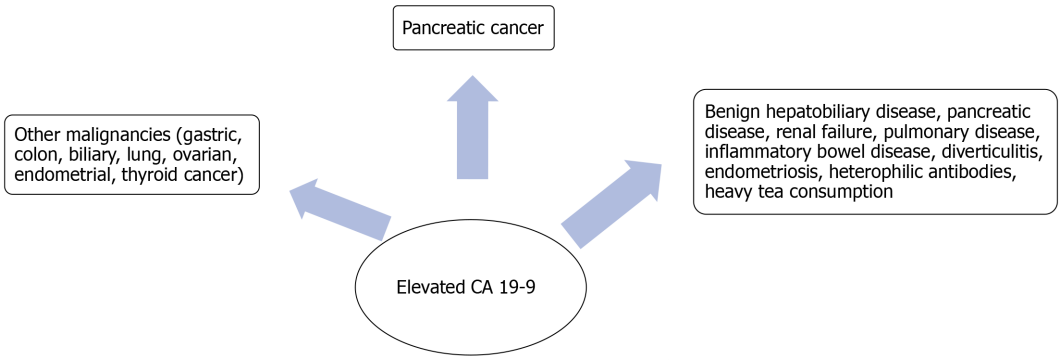

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Figure 1 Causes of elevated carbohydrate antigen 19-9. CA 19-9: carbohydrate antigen 19-9.

patient category[11-13] (Figure 2).

Given the large heterogeneity of PDAC, delineation of subgroups with different tumor biology is considered of paramount importance for personalized management. Currently available literature is inconsistent regarding the clinical features and outcomes of patients with CA 19-9 or Lewis negative PDAC. Some authors have shown a better prognosis, while others have revealed worse outcomes compared to high CA 19-9 PDAC[14,15]. Our aim was to delineate the phenotype of CA 19-9 negative PDAC according to clinical features, disease staging and prognosis as compared with high CA 19-9 PDAC cases.

MATERIALS AND METHODS

Study design and patient population

We performed a retrospective analysis of patients admitted to our Gastroenterology department during a period of 30 mo, from January 2019 to July 2021, who were diagnosed with PDAC by endoscopic ultrasound guided tissue acquisition. Demographic, clinical, laboratory work-up and imaging data were collected from patients' medical records. Staging was carried out based on pancreatic-protocol computed tomography scan, according to the International Association of Pancreatology criteria for resectability-resectable, borderline resectable, locally advanced or metastatic disease[16]. Regarding tumor location, we grouped cases into lesions extended to head, uncinate and neck of the pancreas comprising one set and tumors of the body and tail representing another set. A 6-mo follow-up aimed at assessing survival was carried out either by reaching out to the general practitioner/oncologist or by contacting the patient/patient's family by phone. Patients with missing data according to items assessed in this research were excluded from analysis. Also, patients lost from follow-up were excluded as survival could not be determined.

For the purpose of this study, we divided patients into two groups according to CA 19-9 levels. A threshold was set at 37 U/mL, and patients were classified as CA 19-9 negative or normal (for values < 37 U/mL)-group A and CA 19-9 positive (≥ 37 U/mL)-group B. We then compared the two groups according to demographic and clinical data, biomarkers, tumor staging and 6-mo survival.

Statistical analysis

Data analysis was carried out using SPSS Statistics 25 software (Armonk, NY, United States). Continuous variables were reported as mean, and categorical variables were reported as count and percentage. Comparison among the two groups was done using χ^2 tests for categorical variable and a two-sample *t*-test for continuous variables at a significance of $\alpha = 0.05$.

RESULTS

Altogether 111 patients were analyzed for the purpose of this study; 29 had documented normal CA 19-9 (< 37 U/mL) and 82 were CA 19-9 positive (≥ 37 U/mL). Demographic data, tumor characteristics and outcomes among the two groups was summarized in Table 1.

With regard to sex distribution, a male predominance was seen in the study cohort (75/111, 67.5%), mostly owing to a higher male:female ratio in group B (2.4:1). Median age was similar between the two groups.

Table 1 Characteristics of study patients according to carbohydrate antigen 19-9 value

	Group A (*n* = 29)	Group B (*n* = 82)	*P* value
Patient demographics			
Age in yr, median	64	67	0.241
Male sex	58.62	70.73	0.333
At risk behaviors			
Smoking	31.03	28.04	0.946
Drinker	20.68	23.17	0.987
Clinical findings			
Abdominal pain	55.17	76.83	0.048
Jaundice	27.58	29.26	0.946
Weight loss	62.06	63.41	0.924
Diabetes mellitus	34.48	34.14	0.845
Tumor localization			
Head, neck and uncinate	62.06	57.31	0.820
Body and tail	37.93	42.68	
Tumor size in cm			
< 2	10.34	2.43	0.447
2-4	58.62	64.63	
> 4	31.03	32.92	
Staging			
Resectable	13.79	7.31	0.714
Borderline resectable	6.89	4.87	
Locally advanced	20.68	24.39	
Metastatic	58.62	63.31	
Outcome			
6-mo survival	58.62	47.56	0.308

Data are %, unless otherwise indicated. Group A: Patients classified as CA 19-9 negative or normal (< 37 U/mL); Group B: Patients classified as CA 19-9 positive (≥ 37 U/mL).

Considering at risk behavior among the patient population, a higher proportion of smokers was seen in group A (31.03% *vs* 28.04%), while heavy alcohol consumption was seen slightly more frequently in group B (23.17% *vs* 20.68%). Concerning the symptoms, abdominal pain was more prevalent in patients from group B (76.83% *vs* 55.17%), while weight loss and jaundice were noted in similar proportions in both patient groups. Also, diabetes mellitus was seen in about one-third of patients in both groups (34.48% *vs* 34.14%).

The average value of CA 19-9 was 16904.85 for group B compared with 8.48 for group A. In this latter group of patients, 20.68% had elevated levels for both CEA and CA 125, 13.79%for CA 125 only and 17.24% for CEA only. For both groups analyzed, most tumors (62.06%-group A, 57.31%-group B) were located in the head or uncinate process, while the remaining 37.93% and 42.68%, respectively, developed in the body or tail region. Regarding tumor size, there were no significant differences among the two groups in tumors over 4 cm. A higher proportion of lesions under 2 cm was reported in group A (10.34% *vs* 2.43%), while tumors sized 2-4 cm were more frequently seen in group B (64.63% *vs* 58.62%).

Analysis of tumor staging revealed there were more resectable (13.79% *vs* 7.31%) or borderline resectable tumors (6.89% *vs* 4.87%) in group A, while locally advanced and metastatic tumors were predominant in group B (24.39% *vs* 20.68%, 63.41% *vs* 48.62%). Six-month survival was higher in group A (58.62%) compared to group B (47.56%).

We further performed a subgroup analysis according to sex, taking into account the male predominance of our study cohort. While there were more men with elevated CA 19-9 than women (77.33% *vs* 66.67%), the proportion of locally advanced or metastatic tumors was higher in subgroup B females than

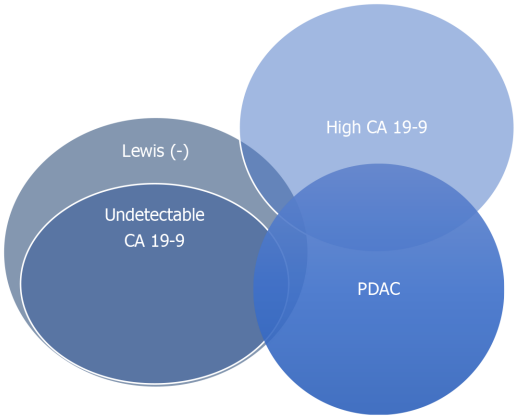

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Figure 2 Interrelation between Lewis phenotype, carbohydrate antigen 19-9 and pancreatic ductal adenocarcinoma. CA 19-9: carbohydrate antigen 19-9; PDAC: Pancreatic ductal adenocarcinoma.

males (95.83% *vs* 84.48%). Regarding symptomatology, abdominal pain was more frequent in group B for both sexes, but the difference seen with group A was higher for males (72.41% *vs* 47.06%) than females (87.50% *vs* 66.67%) without being statistically significant. We also conducted an analysis according to an age threshold set at 65 years. While advanced tumors were seen more in subgroup A less than 65 years of age compared to over 65 (86.7% *vs* 71.4%), in group B 90.5% of elderly patients had locally advanced or metastatic neoplasia compared to 83.9% in those under 65 years. Six-month survival was similar in subgroups A and B according to the 65-year threshold (57.1% and 49.0% for patients under 65 years and 60.0% and 45.2%, respectively, for those 65 years or older).

DISCUSSION

CA 19-9 is the most widely used biomarker for PDAC, but its major drawbacks are represented by false positive results in benign inflammatory conditions and extra-pancreatic neoplasms and by false negative results in Lewis negative individuals, which comprise about 10% of the Caucasian population [5]. However, in several aspects CA 19-9 remains a valuable biomarker for PDAC management, from screening and diagnosis to treatment response, prognosis and recurrence (Figure 3)[9,17-21].

In our study, we enrolled PDAC patients and divided them into two groups: CA 19-9 positive (n = 82) and CA 19-9 negative (n = 29), according to a threshold of 37 U/mL. Six-month survival was better in the CA 19-9 negative patients (58.62% *vs* 47.56%), reflecting a lower proportion of locally advanced and metastatic disease in this group. This could be explained by triggering of imaging studies in patients with elevated CA 19-9, leading to an early stage diagnosis and thus a better prognosis, while in patients with negative CA 19-9 further investigations are often deferred due to lack of concern, leading to delayed diagnosis in advanced stages and poorer prognosis.

Some authors have proposed genotyping Lewis antigen along with CA 19-9 dosing in order to improve its diagnostic accuracy[22,23], but recent studies have shown that CA 19-9 retains its utility even in Lewis negative individuals[11]. CA 19-9 values over 37 U/mL were seen in 27.4% of Lewis negative patients, and areas under the receiver operating characteristic curve for the diagnostic accuracy of CA 19-9 were similar in Lewis negative PDAC patients compared to all PDAC patients (0.842 *vs* 0.898). This was also shown by Kwon *et al*[14], who also found that not all Lewis negative PDAC patients are non-secretors of CA 19-9. In this study, 172/375 (45.87%) of patients in the Le(a−b−) group had a serum CA 19-9 over 37 U/mL. The paradoxical elevation of CA 19-9 in Lewis negative individuals might be explained by partial secretion of the protein, which can be detected by enzymatic immunoassays or by cross-reactivity of the antibodies used for CA 19-9 dosing; treating the collected specimen with blocking agents has been proposed as a method to eliminate interference with heterophilic antibodies[5,13,24]. Therefore, PDAC prognosis is different if patients are stratified according to either CA 19-9 or to Lewis antigen.

A literature search of studies assessing PDAC outcomes according to CA 19-9 and Lewis antigen status has shown inconsistent results (Table 2)[11,14,25-40]. While low CA 19-9 PDAC has been associated with better prognosis, some have shown that Lewis negative PDAC harbors a more aggressive tumor biology and has a poorer outcome[15]. Discordant results might be due to different patient populations and different timeframes of studies, and not least to overlap of Lewis-negative with detectable CA 19-9 PDAC patients. Some authors have concluded that the usefulness of the 37 U/mL threshold for CA 19-9 is more appropriate for PDAC diagnosis than predicting prognosis. However, others have shown a strong correlation of CA 19-9 with tumor burden, survival and recurrence[41,42].

Table 2 Studies reporting on pancreatic ductal adenocarcinoma prognosis according to carbohydrate antigen 19-9 level or Lewis antigen status

Survival analysis according to CA 19-9 values			
Ref.	***n***	**CA 19-9 in U/mL**	**Survival**
			Overall median survival in mo
Berger *et al*[30], 2004	7	Undetectable	32
	21	≤ 37	35
	44	38-200	22
	57	> 200	16
Ferrone *et al*[33], 2006			Mean survival time in yr
	29	< 37	2.3
	82	≥ 37	1.6
Waraya *et al*[28], 2009			Disease-specific survival in mo
	23	≤ 37	30.6
	66	> 37	12.7
Hirakawa *et al*[29], 2011			Median survival in mo
	41	Normal	39.0
	84	Elevated	16.9
Hartwig *et al*[32], 2011			Median survival in mo
	232	< 37	28.0
	418	37-399	23.5
	239	≥ 400	14.5
Turrini *et al*[40], 2009			Median survival in mo
	50	< 37	22
	53	400-900 (n = 27), > 900 (n = 26)	15
Katz *et al*[34], 2010			Median survival in mo
	21	< 37	52.8
	78	> 37	21.2
Kondo *et al*[35], 2010			Preoperative 3-yr survival (%)
	32	< 37	57%
	77	> 37	30%
Hata *et al*[36], 2012			Preoperative median survival in mo
	51	< 37	16.2
	218	> 37	16.4
Bergquist *et al*[37], 2016			Median OS in mo
	3666	< 37	19.1
	7140	> 37	14
Jia *et al*[38], 2019			Median OS in mo
	13	< 35	21
	107	≥ 35	11
Mattiucci *et al*[25], 2019			Median OS in mo
	39	0-5.0	25
	167	5.1-37.0	38

	139	37.1-100.0	32
	178	100.1-353.0	22
	177	> 353.1	20
Kondo *et al*[26], 2017			Median survival in mo
	65	< 37	52.0
	84	≥ 37	23.7
	88	< 50	52.0
	61	≥ 150	20.9
	101	< 300	46.7
	48	≥ 300	18.8
Dong *et al*[27], 2014			Median OS in mo
	18	< 37	21.6
	102	≥ 37	14.2
Kang *et al*[31], 2007			Disease free survival in mo
	18	< 50	22.20
	43	≥ 50	19.31
Kwon *et al*[14], 2020			Median survival in d
	408	< 37	644
	779	> 37	340
Survival analysis according to Lewis antigen status			
Luo *et al*[39], 2017			Median survival in mo
	682	137 CA 19-9 (-)	Stage I, II: 16.6 in Lewis (-), 17.6 in Lewis (+)
		47 Lewis (-)	Stage III, IV: 6.0 in Lewis (-), 7.8 in Lewis (+)
Luo *et al*[11], 2018			Median survival in mo
	1482	19.8% CA 19-9 (-)	8.0 in Lewis (-)
		8.4% Lewis (-)	10.0 in Lewis (+)
Kwon *et al*[14], 2020			Median survival
	1187	203 CA 19-9 (-)	356 d in Lewis (-)
		375 Lewis (-)	477 d in Lewis (+)

CA 19-9: Carbohydrate antigen 19-9; OS: Overall survival.

In order to better predict outcomes, some have proposed measuring other markers such as CA 242, CA 50, CEA, CA 125 or periostin complementary to CA 19-9 for PDAC[43-48]. Additional markers, such as CEMIP, apolipoprotein A-I and transferrin[49,50], were shown to be useful especially in PDAC with normal CA 19-9 levels. Lee *et al*[49] showed that CEMIP (also called KIAA1199) had a diagnostic yield of 86.1% in CA 19-9 negative PDAC, and the combination of CEMIP + CA 19-9 had a significantly improved area under the receiver operating characteristic curve over CA 19-9 alone (0.94 *vs* 0.89, $P < 0.0001$). In our study, 34.47% of CA 19-9 negative PDAC cases had elevated levels of CA 125, 37.92% for CEA and 20.68% for both. Concerning the patients with negative CA 19-9 and positive CA 125 and CEA, 83.33% had metastatic disease at the time of the diagnosis and only 50.00% survived at 6 mo.

Similar results were seen in the paper by Luo *et al*[39]. In Lewis negative patients, high values of CEA were seen in 63.8% of patients, and CA 125 was seen in 51.1%. They concluded that CEA and CA 125 should be routinely measured for PDAC. Considering the metastatic burden and survival among 853 pancreatic cancer patients, Liu *et al*[15] observed that Lewis negative PDAC constitutes an aggressive tumor subtype, with low secretion of CA 19-9 and high secretion of CA125. In line with Luo *et al*[39], others have highlighted the fact that CEA and CA 125, similar to CA 19-9, can also be used to monitor therapeutic response[51].

Interestingly, several papers have shown that CA 19-9 and the other biomarkers are upregulated early in the course of PDAC development-up to 2 years before clinical diagnosis and can be used to

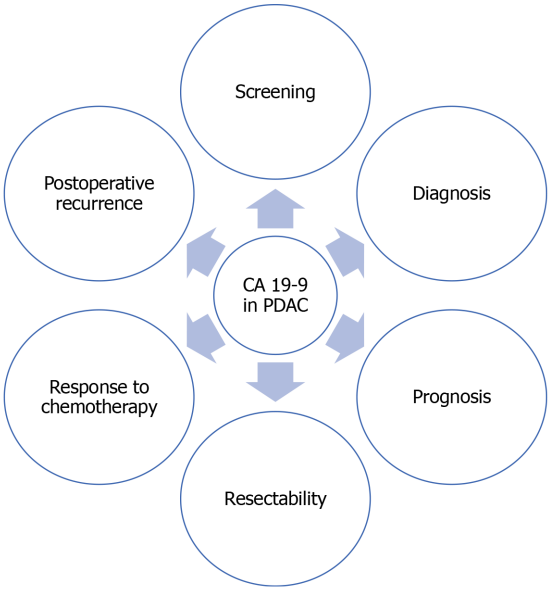

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Figure 3 Usefulness of carbohydrate antigen 19-9 in pancreatic ductal adenocarcinoma management. CA 19-9: carbohydrate antigen 19-9; PDAC: Pancreatic ductal adenocarcinoma.

detect preclinical pancreatic cancer[52,53]. This could be useful for screening strategies of high-risk groups, keeping in mind that Lewis negative individuals might be missed by this approach. Moreover, clinicians should take note that CA 19-9 is also of limited value in the follow-up of Lewis negative patients, in order to avoid erroneous decisions in PDAC management.

The current study has several limitations. Patients recruited in this study were from a hospital-based setting, which had either an acute presentation (jaundice, pancreatitis) or were referred for diagnostic procedures. Also, we acknowledge the lack of Lewis antigen genotyping in our study population, which might have provided further insight into PDAC outcomes according to both CA 19-9 and Lewis antigen status. Another important limitation is the sample size, which makes it very difficult to obtain a statistically significant analysis.

CONCLUSION

In our study, patients with negative CA 19-9 had a better prognosis than those with values over 37 U/mL. Elevated CA 19-9 at diagnosis seems to be associated with a more pronounced symptomatology and higher tumor burden. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

ARTICLE HIGHLIGHTS

Research background

Carbohydrate antigen 19-9 (CA 19-9) is the most widely used biomarker for pancreatic ductal adenocarcinoma (PDAC), but its use is hindered by both false-positive and false-negative results.

Research motivation

There are inconsistent results regarding the outcome of CA 19-9 negative PDAC cases.

Research objectives

To delineate the phenotype of negative CA 19-9 PDAC according to clinical features, disease staging and outcome.

Research methods

Retrospective single-center analysis of PDAC cases over a period of 30 mo.

Research results

Among 111 recruited patients, 29 had normal CA 19-9. Patients with elevated CA 19-9 had higher tumor burden and more advanced staging. Six-month survival was higher for the negative CA 19-9 group (58.62% *vs* 47.56%).

Research conclusions

Negative CA 19-9 PDAC has a better prognosis than PDAC with high CA 19-9 values. CEA and CA 125 can be adjunctive useful markers for PDAC, especially in CA 19-9 negative cases.

Research perspectives

Negative CA 19-9 PDAC cases warrant in-depth analysis of tumor biology to assess if there is indeed a different phenotype of neoplasia.

FOOTNOTES

Author contributions: Balaban DV proposed the research idea; Balaban DV, Marin FS, Manucu G and Zoican A drafted the study design; Jinga M critically reviewed the manuscript; all authors were involved in patient recruitment, data analysis, literature review and drawing of figures and tables and contributed to the initial version of the manuscript.

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ORIGINAL ARTICLE

Retrospective Study

Necessity of neutrophil-to-lymphocyte ratio monitoring for hypothyroidism using nivolumab in patients with cancer

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Abstract

BACKGROUND

Low neutrophil-to-lymphocyte ratio (NLR) has been shown to be associated with a favorable therapeutic response to nivolumab. The activation of immunocompetent cells such as lymphocytes exhibits an antitumor effect; however, the development of excessive immune responses in autologous organs along with the breakdown of self-tolerance causes immune-related adverse events, including hypothyroidism. Therefore, the possibility that NLR is associated with immune response shows that NLR can be not only a predictive factor for good response to nivolumab but also a predictive factor for the development of hypothyroidism.

AIM

To evaluate whether continuous NLR monitoring during nivolumab treatment is useful for predicting the incidence and onset period of hypothyroidism.

METHODS

This retrospective study comprised patients who received nivolumab for treating all types of cancer at our hospital between January 2015 and December 2019. The NLRs of patients were measured before each administration, and the patients were followed up till the administration of 12 doses. NLR at treatment initiation was compared between patients with and without hypothyroidism. Patients who developed hypothyroidism were categorized into three groups: those with NLR < 3.5, 3.5 to < 5, and ≥ 5 according to their maximum NLR from treatment initiation to hypothyroidism development. Further, the onset periods of hypothyroidism were compared between the groups.

RESULTS

Overall, 104 patients were included in the analysis. Twenty-one patients developed hypothyroidism throughout the observation period. NLR at treatment initiation was significantly lower (2.54 ± 1.21 *vs* 4.58 ± 4.03; $P = 0.017$) in patients with hypothyroidism than in those without hypothyroidism, and patients with NLR < 5 had a significantly higher incidence of hypothyroidism than those with NLR ≥ 5 (26%: 20 of 78 patients *vs* 4%: 1 of 26 patients; $P = 0.022$). Additionally, treatment continuity in patients with hypothyroidism was significantly longer than in those without hypothyroidism (median not reached *vs* 7 times administration, $P = 0.010$). Patients with maximum NLR < 3.5 until the development of hypothyroidism had a significantly earlier onset of hypothyroidism than those with maximum NLR ≥ 5 (hazard ratio for low tertile [NLR < 3.5] *vs* high tertile [NLR ≥ 5]: 5.33, $P = 0.011$).

CONCLUSION

Low NLR at treatment initiation increases the incidence of treatment-induced hypothyroidism. Furthermore, its persistence may be a risk factor for the early onset of hypothyroidism.

Key Words: Nivolumab; Hypothyroidism; Immune checkpoint inhibitors; Immune-related adverse event; Neutrophil-to-lymphocyte ratio

Core Tip: This study evaluated whether continuous monitoring of neutrophil-to-lymphocyte ratio (NLR) during nivolumab treatment is useful for predicting the incidence and onset period of hypothyroidism. Patients with hypothyroidism had a significantly lower NLR at treatment initiation, and hypothyroidism incidence was higher among those with NLR < 5. Patients with persistently low NLR (< 3.5) developed hypothyroidism earlier than those with an NLR of 3.5 to < 5 and ≥ 5. Low NLR at treatment initiation increases the incidence of treatment-induced hypothyroidism. Furthermore, its persistence may be a risk factor for the early onset of hypothyroidism.

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INTRODUCTION

The immune checkpoint inhibitor nivolumab restores and activates antigen-specific T cells that have become unresponsive to cancer cells by inhibiting the binding of programmed death-1 (PD-1) to PD-1 Ligands (PD-L1) and exerts antitumor effects[1]. Nivolumab has been successfully used to treat various types of cancer, including advanced melanoma, non-small-cell lung cancer, renal cell carcinoma, classical Hodgkin lymphoma, head and neck cancer, gastric cancer, and malignant pleural mesothelioma. Although nivolumab exerts a remarkable effect on cancer, it requires a certain period until the manifestation of treatment response[2-10]. Considering that other treatments may be required if nivolumab does not achieve a good treatment response, early identification of predictive factors for its efficacy is highly desired. Treatment with nivolumab is accompanied by immune-related adverse events (irAEs), such as hypothyroidism[11]. A recent study suggested that the development of irAEs was associated with treatment benefit[12-15]. The mechanism by which nivolumab elicits an antitumor and antithyroid immune response has not been fully elucidated. The activation of immunocompetent cells by nivolumab results in an antitumor effect. However, the development of excessive immune responses in autologous organs along with the breakdown of self-tolerance causes irAEs, such as hypothyroidism. Neutrophil-to-lymphocyte ratio (NLR) has gained attention as a predictive factor for the efficacy of nivolumab; particularly, low NLR at treatment initiation has been associated with a favorable therapeutic response[16-20]. Therefore, it is assumed that the association of NLR with an immune response shows that NLR is both a predictive factor for nivolumab efficacy and an indicator of the risk for hypothyroidism. In our previous study with patients who responded to six or more doses of nivolumab, we showed that patients with NLR < 5 at the 6th administration had a significantly higher incidence of hypothyroidism[21]. Although we showed the effect of low NLR on the incidence of hypothyroidism, NLR was evaluated only at a fixed observation point, i.e., at the 6th administration of nivolumab. In this study, we investigated whether continuous monitoring of NLRs during nivolumab

treatment is necessary to predict the frequency and onset period of hypothyroidism.

MATERIALS AND METHODS

Patients

This single-center retrospective study comprised patients who received nivolumab regardless of the type of cancer at the Jikei University Hospital between January 2015 and December 2019. The dosage of nivolumab was 3 mg/kg every 2 wk up to October 2018, and due to the revision in guidelines, the dosage of nivolumab was 240 mg/person every 2 wk thereafter. This study included patients who underwent thyroid-stimulating hormone (TSH) and free thyroxine (FT4) measurements at every or alternate administration of nivolumab to assess fluctuation in NLR. The exclusion criteria were as follows: patients with a history of hypothyroidism, thyroid cancer; those at treatment initiation; and those with TSH levels above the upper limit or FT4 Levels below the lower limit of the reference values. Patients who discontinued nivolumab after single administration were also excluded from the analysis because fluctuations in laboratory data could not be analyzed. The reference values of TSH and FT4 Levels were 0.34-4.04 μIU/mL and 0.88-1.67 ng/dL, respectively, based on the Japanese Committee for Clinical Laboratory Standards. In this study, hypothyroidism was defined as TSH levels exceeding the upper limit or FT4 Levels falling below the lower limit of the reference values twice in a row during the nivolumab observation period, with the follow-up period being up to the 12th administration.

NLR and nivolumab treatment continuity

NLR was calculated by dividing the absolute neutrophil and lymphocyte counts measured in peripheral blood samples at each administration. The follow-up period was up to the 12th administration, and each NLR from treatment initiation to the 12th administration was investigated. The decision to discontinue treatment was made by the clinician depending on the progression of disease or the development of severe irAEs. Fluctuations in NLRs were assessed for the following groups of patients: Those who discontinued treatment after administering nivolumab < 6 times, those who discontinued treatment after administering nivolumab 6-11 times, and those who administered nivolumab ≥ 12 times. In particular, we compared NLR fluctuation at treatment initiation and discontinuation among the patients who received nivolumab < 6 times and 6-11 times. Among the patients who received nivolumab ≥ 12 times, we compared NLR fluctuation at treatment initiation and the 12th administration.

Furthermore, we categorized the patients into three groups according to the tertiles of their mean NLR as follows: NLR < 3.5, NLR 3.5 to < 5, and NLR ≥ 5 during the observation points. This analysis compared the differences in treatment continuity between the NLR 3.5 to < 5 and NLR ≥ 5 groups relative to the NLR < 3.5 group.

NLR and hypothyroidism

Patients were classified into two groups according to the presence or absence of hypothyroidism, and the difference in treatment continuity between the two groups was evaluated.

Patients who developed hypothyroidism were categorized into three groups according to the tertiles of their maximum NLR from treatment initiation to development of hypothyroidism as follows: NLR < 3.5, NLR 3.5 to < 5, and NLR ≥ 5. The onset period of hypothyroidism was defined as the number of times nivolumab was administered until the onset. This analysis compared the differences in onset period of hypothyroidism between the NLR < 3.5 and NLR 3.5 to < 5 groups relative to the NLR ≥ 5 group.

Statistical analysis

The distribution of continuous variables was evaluated using the Shapiro-Wilk test. Based on the distribution of the data, continuous variables were statistically analyzed using the Student t test or Mann-Whitney's *U*-test. Categorical variables were statistically analyzed using Fisher's exact test. For comparing the NLR levels during nivolumab treatment or at discontinuation, we used the Wilcoxon signed-rank test for the following groups: patients who discontinued treatment after administering nivolumab < 6 times, those who discontinued treatment after administering nivolumab 6-11 times, and those who administered nivolumab ≥ 12 times. The differences in nivolumab treatment continuity and onset period of hypothyroidism were calculated using the Kaplan-Meier method and analyzed using the log-rank test and Cox proportional hazards analysis. All statistical data were analyzed using the BellCurve for Excel (Social Survey Research Information Co., Ltd. Tokyo, Japan). The significance level of the tests was set at 0.05.

RESULTS

Patients and NLR at treatment initiation

A total of 104 patients were included in the analysis. Nivolumab was administered primarily at 2-week intervals, but it was temporarily administered at 3-week intervals when the hospital was closed or requested by the patient. Table 1 summarizes the background characteristics of patients who received nivolumab and their types of cancers. Throughout the observation period, 21 of 104 (20%) patients developed hypothyroidism. NLR at treatment initiation in patients with hypothyroidism was significantly lower than that in patients without hypothyroidism (2.54 ± 1.21 *vs* 4.58 ± 4.03; $P = 0.017$). Patients with NLR < 5 had a significantly higher incidence of hypothyroidism than those with NLR ≥ 5 (26%: 20 of 78 patients *vs* 4%: 1 of 26 patients; $P = 0.022$).

Association between NLR and nivolumab treatment continuity

The median values of NLR at treatment initiation in patients who received nivolumab administration < 6, 6-11, and ≥ 12 times were 4.01, 3.03, and 2.64, respectively (Figure 1). A significant increase in NLR was observed at discontinuation in 40 patients who discontinued treatment after administering nivolumab < 6 times (median NLR, 4.01 *vs* 5.92, $P = 0.020$; Figure 1A). The reasons for the discontinuation of nivolumab in these patients were progression of disease in 34 patients and development of severe irAEs in six patients (pneumonitis: two patients, rashes: one patient, myocarditis: one patient, hypophysitis: one patient, and eosinophilia: one patient). A significant increase in NLR was observed at discontinuation in 32 patients who discontinued treatment after administering nivolumab 6-11 times (median NLR, 3.03 *vs* 3.50, $P = 0.038$; Figure 1B). The reasons for the discontinuation of nivolumab in these patients were progression of disease in 26 patients and severe irAEs in six patients (pneumonitis: three patients, rashes: two patients, and colitis: one patient). Finally, no significant differences in NLR were observed between the treatment initiation and the 12th administration in 32 patients who received nivolumab ≥ 12 times (median NLR, 2.64 *vs* 2.32, $P = 0.940$; Figure 1C).

When the population was categorized into three groups based on the tertiles of their mean NLR during the observation period as NLR < 3.5, 3.5 to < 5, and ≥ 5, we observed a significant difference in treatment continuity between the three groups, as shown in Figure 2. The median number of times that nivolumab was administered in each group with mean NLR < 3.5, 3.5 to < 5, and ≥ 5 was 11.5, 8, and 4, respectively. The groups with mean NLR < 3.5 and 3.5 to < 5 had significantly longer treatment continuity than the group with NLR ≥ 5 (hazard ratio [HR] for low tertile compared with high tertile: 0.23; 95% confidence interval [CI]: 0.13-0.41, $P < 0.001$; HR for middle tertile compared with high tertile: 0.32; 95%CI: 0.17-0.60; $P < 0.001$).

Association between NLR and hypothyroidism

Treatment continuity was significantly longer in patients who developed hypothyroidism than in patients without hypothyroidism (median not reached *vs* 7 times administration, $P = 0.010$; Figure 3).

No patients discontinued nivolumab due to hypothyroidism. In patients who developed hypothyroidism, the reasons for discontinuing nivolumab during the observation period were progression of disease in nine patients and severe irAEs in two patients (pneumonitis: one patient and rashes: one patient). In patients without hypothyroidism, the reasons for discontinuing nivolumab during the observation period were progression of disease in 51 patients and severe irAEs in ten patients (pneumonitis: four patients, rashes: two patients, myocarditis: one patient, colitis: one patient, eosinophilia: one patient, and hypophysitis: One patient).

When the population was categorized into three groups based on the tertiles of their maximum NLR from treatment initiation to development of hypothyroidism, we observed a significant difference in the onset period, as shown in Figure 4. The median onset periods of each group with maximum NLRs of < 3.5, 3.5 to < 5, and ≥ 5 were at 5th, 6th, and 9th administrations, respectively. The groups with maximum NLR < 3.5 had a significantly earlier onset of hypothyroidism than the group with NLR ≥ 5, whereas there was no significant difference in the onset periods of the groups with maximum NLRs of 3.5 to < 5 and ≥ 5 (HR for low tertile compared with highest tertile: 5.33; 95%CI: 1.47-19.33, $P = 0.011$; HR for middle tertile compared with highest tertile: 3.15; 95%CI: 0.83-11.89, $P = 0.091$).

DISCUSSION

This study evaluated treatment outcomes as the number of times of nivolumab administration. The median values of NLR at treatment initiation in patients who administered nivolumab < 6, 6-11, and ≥ 12 times were 4.01, 3.03, and 2.64, respectively. Previous studies have found that low NLR at treatment initiation is associated with favorable therapeutic outcomes[16-20]; the results of this study are similar to those previously reported. Because the cancer treatment response to nivolumab is assessed up to the 6th administration[2-10], patients who discontinue after administering nivolumab < 6 times are considered to show a lack of therapeutic effect, whereas those who discontinue after administering nivolumab 6-11 and ≥ 12 times are considered to show a therapeutic effect. Therefore, patients with high NLR at

Table 1 Characteristics of patients at treatment initiation

	All patients (*n* = 104)	Hypothyroidism		
		Yes (*n* = 21)	No (*n* = 83)	*P* value
Male/female	69/35	14/7	55/28	1.000
Median age (min-max) (years)	68.5 (32-91)	70.0 (45-91)	68.0 (32-88)	0.382
Body weight (kg)	52.7 ± 11.9	54.0 ± 9.6	52.4 ± 12.4	0.340
Cancer type				
Head and neck cancer	29	6	23	1.000
Non-small-cell lung cancer	29	6	23	1.000
Malignant melanoma	16	1	15	0.183
Renal cell cancer	15	4	11	0.497
Gastric cancer	15	4	11	0.497
Laboratory data				
TSH (μIU/mL)	2.08 ± 0.80	2.34 ± 0.78	2.02 ± 0.79	0.058
FT3 (pg/mL)	2.21 ± 0.50	2.12 ± 0.32	2.23 ± 0.54	0.584
FT4 (ng/dL)	1.18 ± 0.20	1.11 ± 0.20	1.20 ± 0.19	0.064
NLR	4.17 ± 3.73	2.54 ± 1.21	4.58 ± 4.03	0.017
NLR < 3.5	60	16	44	0.082
NLR ≥ 3.5	44	5	39	
NLR < 5	78	20	58	0.022
NLR ≥ 5	26	1	25	

TSH: Thyroid-stimulating hormone; FT3: Free triiodothyronine; FT4: Free thyroxine; NLR: Neutrophil-to-lymphocyte ratio; min: Minimum; max: Maximum.

treatment initiation may not show a therapeutic effect until the 6th administration, increasing the possibility of discontinuation.

A previous study reported that low NLR at the 4th administration of nivolumab was associated with prolongation in overall survival and that responding patients showed a decline in their longitudinal NLR over time[22,23]. We found that patients with mean NLR < 3.5 and 3.5 to < 5 had significantly longer treatment continuity than those with mean NLR ≥ 5. Thus, we suggest that low NLR (mean NLR < 5) can be useful for predicting treatment continuity. Interestingly, a significant increase in NLR was observed at treatment discontinuation (Figure 1A-C). PD-1 expressed on activated T cells binds to PD-L1 expressed on cancer cells to transmit an inhibitory signal to T cells; however, nivolumab promotes the reactivation of the immune response by suppressing this inhibitory signal[1,24]. Thus, low NLR levels indicates that the antitumor effect of nivolumab sustains the lymphocyte-dominant immune state, whereas an increase in NLR indicates that the weakened immune activation affects the discontinuation of nivolumab.

Patients who developed irAEs have shown favorable treatment response to nivolumab[12,13]. Furthermore, it has recently been reported that patients who developed hypothyroidism, one of the irAEs, during treatment also showed a favorable therapeutic response[14,15]. Our study showed that patients with hypothyroidism have a longer treatment continuity than those without hypothyroidism, supporting the results of the previous studies.

Although it has been mentioned above that monitoring NLR fluctuations during treatment is useful for predicting the therapeutic effect, whether NLR fluctuations can be used to predict the onset period of hypothyroidism is an interesting topic. However, Matsukane *et al*[25] showed that there was no significant change in NLR from the period of treatment initiation to development of hypothyroidism in patients who developed hypothyroidism after administering nivolumab. Thus, NLR fluctuations during treatment cannot predict the development of hypothyroidism. However, the present study revealed that patients who developed hypothyroidism showed significantly lower NLR at treatment initiation and patients with NLR < 5 showed a significantly higher incidence of hypothyroidism than those with NLR ≥ 5. We further investigated whether the persistence of low NLR affected the difference in the onset period of hypothyroidism. In particular, we investigated whether patients with NLR < 3.5 and NLR 3.5 to < 5 at treatment initiation had an earlier onset period than those with NLR ≥ 5. This study showed

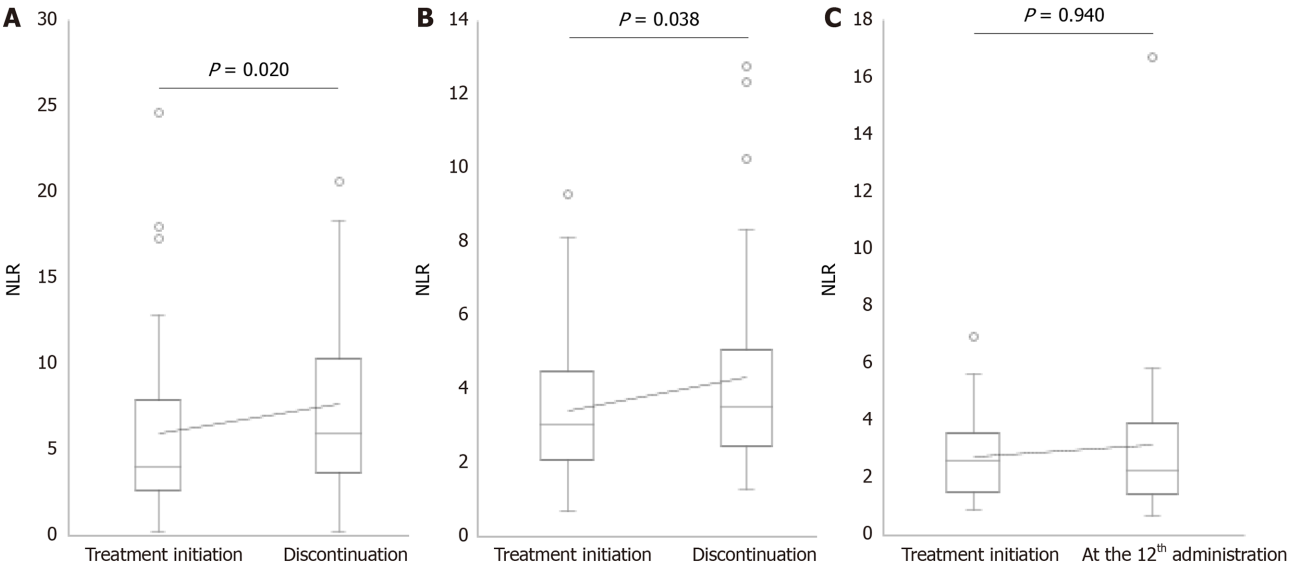

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Figure 1 Neutrophil-to-lymphocyte ratio fluctuation in patients who discontinued treatment after administering nivolumab < 6 times, who discontinued treatment after administering nivolumab 6-11 times, and who administered nivolumab ≥ 12 times. A: A significant increase in neutrophil-to-lymphocyte ratio (NLR) was observed at the discontinuation (n = 40, median NLR = 4.01 *vs* 5.92, P = 0.020); B: A significant increase in NLR was observed at the discontinuation (n = 32, median NLR = 3.03 *vs* 3.50, P = 0.038); C: No significant difference in NLR was observed between treatment initiation and the 12th administration (n = 32, median NLR = 2.64 *vs* 2.32, P = 0.940). NLR: Neutrophil-to-lymphocyte ratio.

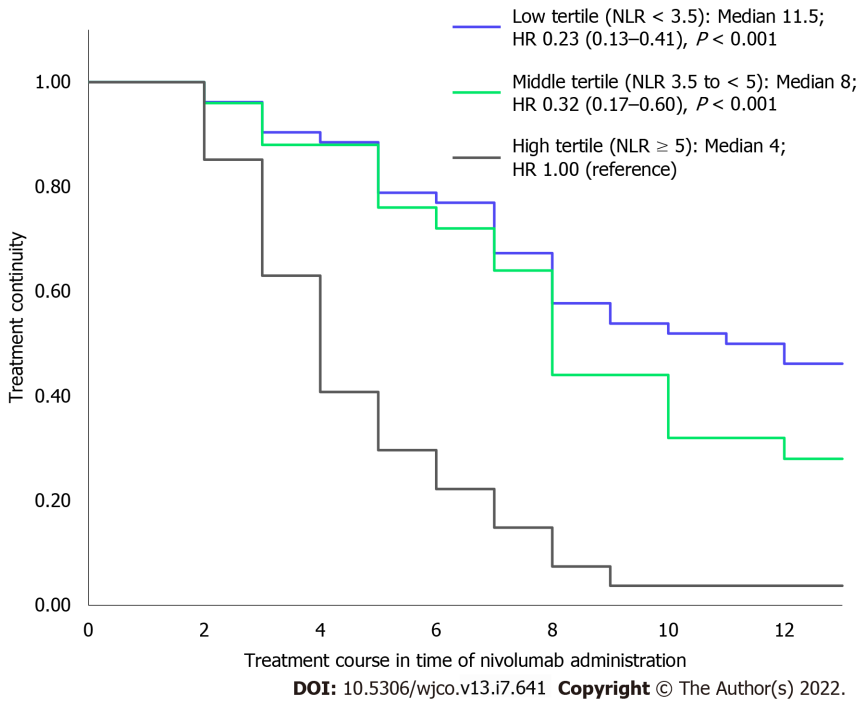

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Figure 2 Relationship between neutrophil-to-lymphocyte ratio and nivolumab treatment continuity. The median numbers of nivolumab administration in each group with mean neutrophil-to-lymphocyte ratio (NLR) < 3.5, 3.5 to < 5, and ≥ 5 were 11.5 (n = 52), 8 (n = 25), and 4 (n = 27), respectively. The groups with mean NLR < 3.5 and 3.5 to < 5 had significantly higher treatment continuity than those with mean NLR ≥ 5 (hazard ratio [HR] for low tertile compared with high tertile: 0.23; 95% confidence interval [CI]: 0.13-0.41, P < 0.001; HR for middle tertile compared with high tertile: 0.32; 95%CI: 0.17-0.60; P < 0.001).

that patients with maximum NLR of < 3.5 until the development of hypothyroidism had a significantly earlier onset of hypothyroidism than those with NLR ≥ 5. Thus, persistently low NLR may be a risk factor for the early development of hypothyroidism. Monitoring the maximum NLR using a cutoff value of < 3.5 as a reference is clinically helpful in predicting the early onset of hypothyroidism.

This study has several limitations. First, this was a retrospective study conducted at a single institution, and the cancer types of patients were not specified. Additionally, there was a bias in cancer

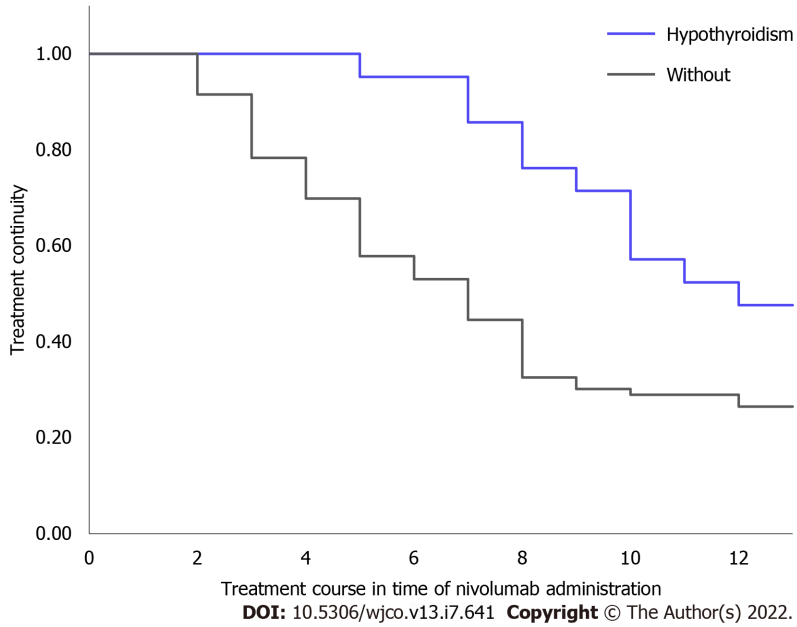

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Figure 3 Nivolumab treatment continuity in patients who developed hypothyroidism. Treatment continuity in patients who developed hypothyroidism was significantly longer than in those who did not develop hypothyroidism (n = 104, median not reached *vs* 7 times administration, P = 0.010).

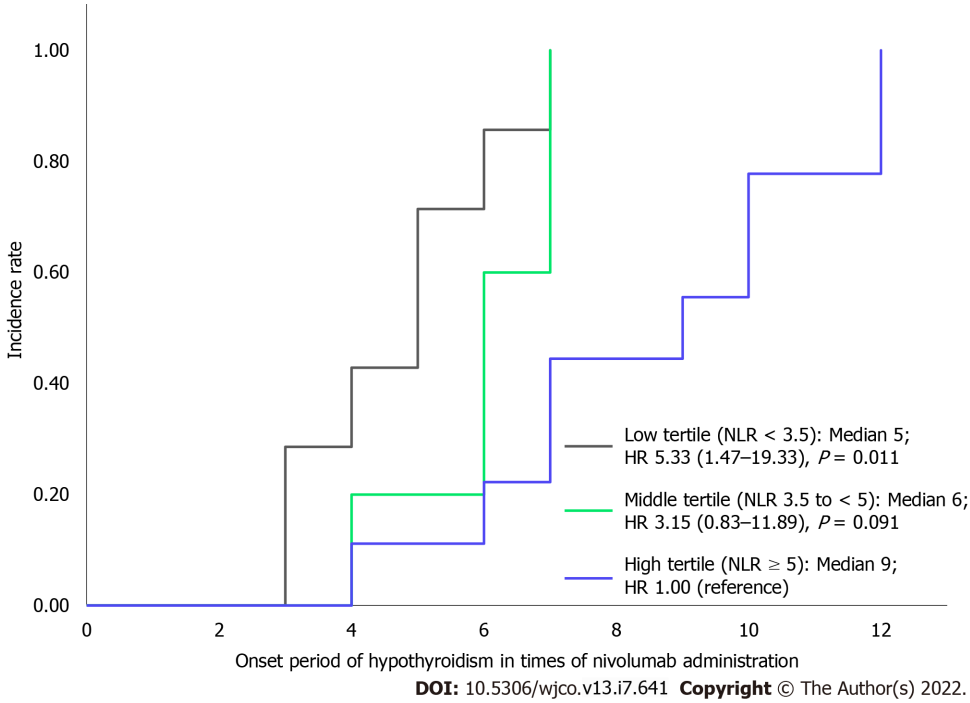

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Figure 4 Relationship between neutrophil-to-lymphocyte ratio and the onset period of hypothyroidism. The median onset periods of each group with maximum neutrophil-to-lymphocyte ratio (NLR) values of < 3.5, 3.5 to < 5, and ≥ 5 were at 5th (n = 7), 6th (n = 5), and 9th administration (n = 9), respectively. The groups with a maximum NLR of < 3.5 had a significantly earlier onset of hypothyroidism than the group with NLR ≥ 5, whereas there was no significant difference in the onset periods of the groups with maximum NLR values of 3.5-5 and ≥ 5 (HR for low tertile compared with highest tertile: 5.33; 95%CI: 1.47-19.33, P = 0.011; HR for middle tertile compared with highest tertile: 3.15; 95%CI: 0.83-11.89, P = 0.091).

types of the patient population. Second, due to the limited sample size of this study population, follow-up with larger populations is needed for verification. Third, the follow-up period was limited to the 12th dose of nivolumab. In fact, in some patients, hypothyroidism develops after 12 doses; hence, the incidence of hypothyroidism should be evaluated throughout the treatment period. Fourth, we analyzed the treatment continuity of nivolumab rather than its therapeutic response as a criterion of therapeutic effect. Further studies are needed on NLR fluctuations *via* treatment response.

The involvement of antithyroid peroxidase antibody or antithyroglobulin antibody has been shown as a factor related to the development of hypothyroidism[26]. However, these laboratory data are not measured regularly in daily clinical practice. Alternatively, as the neutrophil and lymphocyte counts are regularly measured, the possibility of using NLR as a predictive factor was considered to be useful for the evaluation of the treatment continuity of nivolumab and associated adverse effects.

CONCLUSION

Low NLR at treatment initiation increased the incidence of treatment-induced hypothyroidism. Low NLR levels were also associated with the treatment continuity of nivolumab. Thus, the persistence of low NLR may be a risk factor for the early development of hypothyroidism.

ARTICLE HIGHLIGHTS

Research background

The activation of immunocompetent cells by nivolumab exerts an antitumor effect. However, excessive immune responses developed in autologous organs along with the breakdown of self-tolerance causes immune-related adverse events (irAEs), such as hypothyroidism.

Research motivation

Low neutrophil-to-lymphocyte ratio (NLR) values have been shown to be associated with a favorable therapeutic response to nivolumab. The possibility that NLR is associated with immune response implies that NLR can be not only a predictive factor for good response to nivolumab but also a predictive factor for the development of hypothyroidism.

Research objectives

To evaluate whether continuous monitoring of NLRs during nivolumab treatment is useful for predicting the incidence and onset period of hypothyroidism.

Research methods

NLR of patients who received nivolumab treatment was measured before each administration. NLR at treatment initiation was compared between patients with and without hypothyroidism during the treatment period. Patients who developed hypothyroidism were categorized into three groups as those with NLR < 3.5, NLR 3.5 to < 5, and NLR ≥ 5 according to their maximum NLR from treatment initiation to hypothyroidism development, and the onset periods of hypothyroidism were compared.

Research results

Patients with hypothyroidism showed significantly lower NLR at treatment initiation, and the incidence of hypothyroidism was higher among those with NLR < 5. Patients with persistently low NLR (< 3.5) developed hypothyroidism earlier than those with NLR 3.5 to < 5 and NLR ≥ 5.

Research conclusions

Low NLR at treatment initiation increases the incidence of treatment-induced hypothyroidism. Moreover, its persistence may be a risk factor for the early onset of hypothyroidism.

Research perspectives

The follow-up period in this study was limited to the 12th dose of nivolumab. The incidence of hypothyroidism should be evaluated throughout the treatment period.

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FOOTNOTES

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ORIGINAL ARTICLE

Retrospective Study

Assessing radiation dose for postoperative radiotherapy in prostate cancer: Real world data

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Abstract

BACKGROUND

Approximately 30% of patients with localized prostate cancer (PCa) who undergo radical prostatectomy will develop biochemical recurrence. In these patients, the only potentially curative treatment is postoperative radiotherapy (PORT) with or without hormone therapy. However, the optimal radiotherapy dose is unknown due to the limited data available.

AIM

To determine whether the postoperative radiotherapy dose influences biochemical failure-free survival (BFFS) in patients with PCa.

METHODS

Retrospective analysis of patients who underwent radical prostatectomy for PCa followed by PORT-either adjuvant radiotherapy (ART) or salvage radiotherapy (SRT)-between April 2002 and July 2015. From 2002 to 2010, the prescribed radiation dose to the surgical bed was 66-70 Gy in fractions of 2 Gy; from 2010 until July 2015, the prescribed dose was 70-72 Gy. Patients were grouped into three categories according to the total dose administered: 66-68 Gy, 70 Gy, and 72 Gy. The primary endpoint was BFFS, defined as the post-radiotherapy prostate-specific antigen (PSA) nadir + 0.2 ng/mL. Secondary endpoints were overall survival (OS), cancer-specific survival (CSS), and metastasis-free survival (MFS; based on conventional imaging tests). Treatment-related genitourinary (GU) and gastrointestinal (GI) toxicity was evaluated according to Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer

criteria. Finally, we aimed to identify potential prognostic factors. BFFS, OS, CSS, and MFS were calculated with the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression models were performed to explore between-group differences in survival outcome measures.

RESULTS

A total of 301 consecutive patients were included. Of these, 93 (33.6%) received ART and 186 (66.4%) SRT; 22 patients were excluded due to residual macroscopic disease or local recurrence in the surgical bed. In this subgroup (n = 93), 43 patients (46.2%) were Gleason score (GS) ≤ 6, 44 (47.3%) GS 7, and 6 (6.5%) GS ≥ 8; clinical stage was cT1 in 51 (54.8%), cT2 in 35 (39.3%), and cT3 in one patient (1.1%); PSA was < 10 ng/mL in 58 (63%) patients, 10-20 ng/mL in 28 (30.6%), and ≥ 20 ng/mL in 6 (6.4%) patients. No differences were found in BFFS in this patient subset versus the entire cohort of patients (P = 0.66). At a median follow-up of 113 months (range, 4-233), 5- and 10-year BFFS rates were 78.8% and 73.7%, respectively, with OS rates of 93.3% and 81.4%. The 5-year BFFS rates in three groups were as follows: 69.6% (66-68 Gy), 80.5% (70 Gy) and 82.6% (72 Gy) (P = 0.12):the corresponding 10-year rates were 63.9%, 72.9%, and 82.6% (P = 0.12), respectively. No significant between-group differences were observed in MFS, CSS, or OS. On the univariate analysis, the following variables were significantly associated with BFFS: PSA at diagnosis; clinical stage (cT1 *vs* cT2); GS at diagnosis; treatment indication (ART *vs* SRT); pre-RT PSA levels; and RT dose 66 -68 Gy vs. 72 Gy (HR: 2.05; 95%CI: 1.02-4.02, P = 0.04). On the multivariate analysis, the following variables remained significant: biopsy GS (HR: 2.85; 95%CI: 1.83-4.43, P < 0.001); clinical stage (HR: 2.31; 95%CI: 1.47-4.43, P = 0.01); and treatment indication (HR: 4.11; 95%CI: 2.06-8.17, P < 0.001). Acute grade (G) 1 GU toxicity was observed in 11 (20.4%), 17 (19.8%), and 3 (8.3%) patients in each group (66-68 Gy, 70 Gy and 72 Gy), respectively (P = 0.295). Acute G2 toxicity was observed in 2 (3.7%), 4 (4.7%) and 2 (5.6%) patients, respectively (P = 0.949). Acute G1 GI toxicity was observed in 16 (29.6%), 23 (26.7%) and 2 (5.6%) patients in each group, respectively (P = 0.011). Acute G2 GI toxicity was observed in 2 (3.7%), 6 (6.9%) and 1 (2.8%) patients, respectively (P = 0.278). No cases of acute G3 GI toxicity were observed.

CONCLUSION

The findings of this retrospective study suggest that postoperative radiotherapy dose intensification in PCa is not superior to conventional radiotherapy treatment.

Key Words: Prostate cancer; Postoperative radiotherapy; Dose intensified; Radiation dose; Biochemical relapse free survival

Core Tip: This retrospective study was performed to evaluate whether higher doses of postoperative radiotherapy influence biochemical failure-free survival rates in patients with prostate cancer. Our results show no significant differences in biochemical failure-free survival, cancer-specific survival, metastasis-free survival, or overall survival regardless of the radiotherapy dose (66-68 *vs* 70 *vs* 72 Gy). No differences in treatment-related toxicity were observed. These findings suggest that radiation dose intensification is not superior to conventional radiotherapy treatment.

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INTRODUCTION

In the year 2020, prostate cancer (PCa) was the 4th most common cancer worldwide, with an annual incidence of 1414259 cases, and the 8th leading cause of cancer mortality, with 375304 deaths[1]. Radical prostatectomy (RP) is one of the primary treatments for localized PCa, with good long-term results[2]. However, up to 30% of surgically-treated patients will develop biochemical recurrence, which is primarily observed in patients who present high-risk factors in the surgical specimen, positive surgical margins, Gleason score (GS) ≥ 8, extracapsular extension, and/or involvement of the seminal vesicles[3].

In this clinical context, the main international clinical guidelines recommend postoperative radiotherapy (PORT)[4]. There are two main treatment modalities for PORT, adjuvant radiotherapy (ART) or salvage radiotherapy (SRT). ART is defined as the prophylactic administration of RT after RP but before recurrence (when prostate-specific antigen [PSA] levels remain undetectable) in patients with a high risk of recurrence due to adverse pathologic features. By contrast, SRT involves the administration of RT to the prostate bed in patients with confirmed biochemically-recurrent PCa (without evidence of distant metastasis) after surgery[5].

Despite the recent publication of several studies[6-9], the optimal timing of PORT (*i.e.*, ART *vs* SRT) remains unclear in some patient subgroups. The optimal dose for both ART and SRT has not been established, nor is it clear whether dose escalation is appropriate in these patients. Although several studies suggest that dose intensification may be more effective than conventional doses in terms of biochemical control[10-15], other studies have found that dose intensification does not provide any benefits compared to conventional dosing and is also associated with greater toxicity[16].

In this context, the aim of the present retrospective study was to describe long-term clinical outcomes and treatment-related toxicity (acute and chronic) according to the PORT dose (66-68 Gy, 70 Gy, and 72 Gy) in patients treated at our hospital between 2002 to 2015.

MATERIALS AND METHODS

This was a retrospective analysis of patients with PCa who underwent radical prostatectomy followed by PORT (ART or SRT) at the Ramón y Cajal University Hospital in Spain between April 2002 and July 2015. From 2002 to 2010, the dose to the surgical bed was 66-70 Gy; in 2011, the dose was increased to 70-72 Gy. In all cases, the doses were delivered in fractions of 2 Gy, 5 d a week according to the protocol established in that centre at that time and the clinical criteria of the radiation oncology specialist.

Treatment planning was performed with the patient in the supine position, with a full bladder and empty rectum. Contouring of the surgical bed was performed in accordance with Radiation Therapy Oncology Group guidelines[17,18]. Until April 2006, three-dimensional (3D) conformal radiotherapy was used. Thereafter, patients were treated with intensity-modulated radiotherapy (IMRT).

Follow-up was performed by specialists from the Radiotherapy Oncology or Urology Departments at our hospital. The first follow-up visit, consisting of a clinical evaluation and PSA determination, was conducted three months after treatment completion. Subsequent visits were performed every 3-6 mo during the first five years and annually thereafter.

Acute toxicity was defined as any toxicity from the start of radiotherapy until six months after treatment finalisation. Treatment-related toxicity observed > six months after treatment completion was defined as chronic toxicity.

The primary aim of this study was to evaluate BFFS, defined as the PSA nadir + 0.2 ng/mL after completion of RT. Patients were classified into three groups according to the total radiotherapy dose administered to the surgical bed (66-68 Gy, 70 Gy, and 72 Gy). Secondary objectives were as follows: overall survival (OS), cancer-specific survival (CSS), and metastasis-free survival (MFS)-assessed by conventional imaging tests (computed tomography [CT] and bone scan); and genitourinary (GU) and gastrointestinal (GI) toxicity according to Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria[19]. Finally, we evaluated the following variables as potential prognostic factors: PSA level prior to the start of RT; clinical and pathological stage; GS; margin status; radiotherapy dose; hormonal therapy; perineural invasion and treatment indication (ART *vs* SRT).

Statistical analysis was performed with the SPSS statistical software, v.20 (IBM-SPSS Corp). BFFS, OS, CSS and MFS were calculated from the start of RT, using the Kaplan-Meier method and the log-rank test with a significance level of $P < 0.05$. Univariate and multivariate Cox regression models were performed to explore between-group differences in survival measures.

RESULTS

We evaluated 301 consecutively-treated patients. Of these, 93 (33.6%) received ART (≤ six months after surgery) due to unfavourable histological factors (involved or close margins or stage pT3b-T4). A total of 186 patients (66.4%) were treated with SRT after biochemical recurrence. Twenty-two patients were excluded due to residual macroscopic disease or local recurrence in the surgical bed. Lymph node dissection was performed simultaneously with radical prostatectomy in 135 patients (48.6%). The clinicopathologic characteristics of the patients by radiotherapy dose to the surgical bed are shown in Table 1.

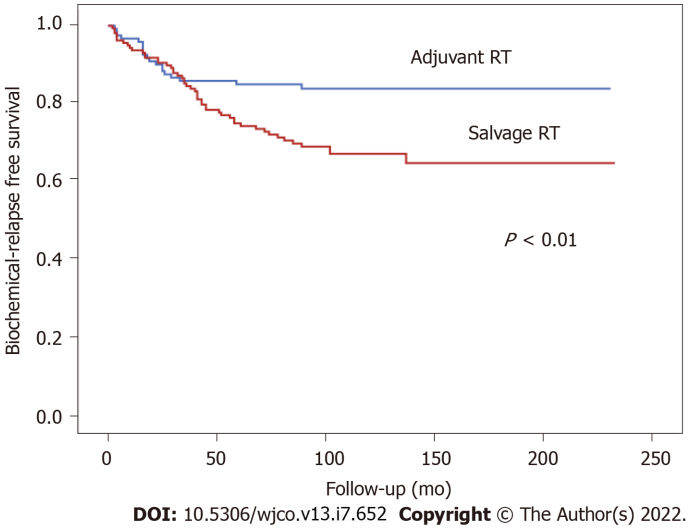
At a median follow-up of 113 mo (range, 4-233), 5- and 10-year survival rates, respectively, were as follows: BFFS: 78.8% and 73.7%; OS: 93.3% and 81.4%; CSS: 95.9% and 88.4%; and MFS: 96.8% and 91.8%. Local recurrence in the surgical bed was observed in four cases (1.5%), lymph node recurrence in 22 patients (8.3%), and distant metastases in 27 patients (10.1%).

Table 1 Clinicopathologic characteristics of the patients according to the radiotherapy dose to the surgical bed

	Total dose			*P* value
	66-68 Gy	70 Gy	72 Gy	
Variable	*n* (%)			
Patients, *n* (%)	59 (21.1)	131 (50)	89 (31.9)	
Median age, range	63.3 (50-74)	62.7 (48-75)	62.3 (41-75)	
PSA preRT				0.36
≤ 0.4 ng/mL	26 (44.1%)	59 (48.8%)	49 (55.7%)	
>0.4 ng/mL	33 (55.9%)	62 (51.2%)	39 (44.3%)	
cT stage				0.43
cT1	38 (67.9%)	65 (57%)	53 (66.2%)	
cT2	18 (32.1%)	46 (40.4%)	26 (32.5%)	
cT3	0 (0%)	3 (2.6%)	1 (1.2%)	
pT stage				0
pT1-T2	45 (80.4%)	94 (75.2%)	41 (47.1%)	
pT3-T4	11 (19.6%)	31 (24.8%)	46 (52.9%)	
pN stage				0.32
N0	25 (42.4%)	60 (46.9%)	42 (48.3%)	
N1	1 (1.7%)	5 (3.9%)	1 (1.1%)	
Nx	33 (55.9%)	63 (49.2%)	44 (50.6%)	
GS (biopsy)				0.06
≤ 6	31 (53.4%)	67 (52.8%)	32 (37.6%)	
7	25 (43.1%)	53 (41.7%)	42 (49.4%)	
≥ 8	2 (3.4%)	7 (5.5%)	11 (12.9%)	
GS (prostatectomy)				0.01
≤ 6	7 (17.9%)	28 (29.5%)	9 (12.2%)	
7	27 (69.2%)	49 (51.6%)	41 (55.4%)	
≥ 8	5 (12.8%)	18 (18.9%)	24 (32.4%)	
Margin status				0.81
Positive	33 (55.9%)	74 (56.9%)	54 (60.7%)	
Negative	26 (44.1%)	56 (43.1%)	35 (39.3%)	
Hormonotherapy				0
Yes	28 (48.3%)	50 (40.3%)	15 (18.1%)	
No	30 (51.7%)	74 (59.7%)	68 (81.9%)	
RT indication				0.68
Adjuvant RT	19 (32.2%)	41 (31.5%)	33 (37.1%)	
Salvage RT	40 (67.8%)	90 (68.5%)	56 (62.9%)	

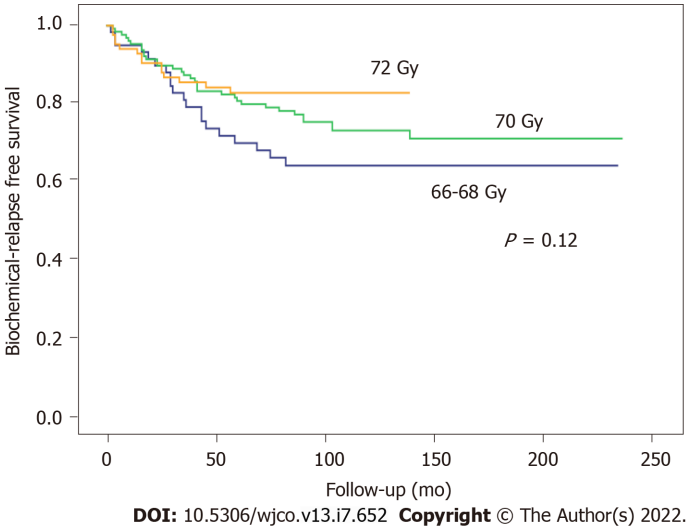
GS: Gleason score; preRT: Pre-radiotherapy; PSA: Prostate-specific antigen

At 5 and 10 years, BFFS was 89.1% and 89.1% in the ART group *vs* 73.3% and 65.5%, respectively, in the SRT group (Figure 1). By total dose, the median BFFS (Figure 2) was not reached in any of the subgroups; the 5- and 10-year BFFS rates in these three groups were 69.6%, 80.5% and 82.6% (P = 0.12) and 63.9%, 72.9% and 82.6% (P = 0.12), respectively; the 5- and 10-year CSS rates in these three groups were 100%, 98.4% and 98.8% and 89.3%, 96.4% and 97.3% (P = 0.067), respectively; the 5- and 10-year OS rates in these three groups were 93.1%, 94.5% and 91.5% and 76.6%, 81.3% and 88.9% (P = 0.519),



DOI: 10.5306/wjco.v13.i7.652

Figure 1 Biochemical relapse free survival according to radiotherapy indication (adjuvant versus salvage radiotherapy). RT: Radiotherapy.



DOI: 10.5306/wjco.v13.i7.652

Figure 2 Biochemical failure-free survival according to radiotherapy dose to the surgical bed.

respectively, Figures 3 and 4.

On the univariate analysis, the following variables were significantly associated with BFFS: PSA at diagnosis (hazard ratio [HR]: 1.05; 95% confidence interval [CI]: 1.77-5.11, $P = 0.00$); clinical stage cT1 *vs* cT2 (HR: 3.01; 95%CI: 1.67-4.75, $P < 0.001$); GS at diagnosis 6 *vs* 7 (HR: 2.31; 95%CI: 1.31-4.08, $P = 0.004$) and 6 *vs* 8-9 (HR: 7.88; 95%CI: 3.76-16.52, $P < 0.001$); (ART *vs* SRT; HR: 3.40; 95%CI: 1.74-6.66, $P = 0.00$); PSA level prior to RT (HR: 1.25; 95%CI: 1.14-1.38, $P < 0.001$); and RT dose 66-68 Gy *vs* 72 Gy (HR: 2.05; 95%CI: 1.02-4.02, $P = 0.04$). None of the following variables were associated with BFFS: preoperative androgen blockade ($P = 0.66$), perineural invasion ($P = 0.15$), or involved margins ($P = 0.36$).

On the multivariate Cox regression analysis, the following variables remained significantly associated with BFFS: GS in the biopsy (HR: 2.85; 95%CI: 1.83-4.43, $P < 0.001$); clinical stage (HR: 2.31; 95%CI: 1.47-3.43, $P = 0.01$); and the indication for external beam radiation therapy (ART *vs* SRT), (HR: 4.11; 95%CI: 2.06-8.17, $P < 0.001$).

On the univariate analysis, the following variables were significantly associated with OS: Age (HR: 1.07; 95%CI: 1.02-1.12, $P = 0.003$); GS in the surgical specimen: GS 6 *vs* 8-9 (HR: 2.36; 95%CI: 1.01-5.52, $P = 0.048$); PSA prior to RT: ≤ 4 *vs* > 4 ng/mL (HR: 1.81; 95%CI: 1.07-3.06, $P = 0.027$); and distant metastases (HR: 2.49; 95%CI: 1.37-4.53, $P = 0.003$). On the multivariate analysis, only age (HR: 1.09; 95%CI: 1.03-1.13, $P = 0.002$) and distant metastases (HR: 2.82; 95%CI: 1.54-5.16, $P = 0.001$) remained significant.

Acute grade (G)1 GU toxicity was observed in 11 (20.4%), 17 (19.8%), and 3 (8.3%) patients in each group (66-68 Gy, 70 Gy, and 72 Gy), respectively, ($P = 0.295$). Acute G2 GU toxicity was observed in 2 (3.7%), 4 (4.7%) and 2 (5.6%) patients, respectively, ($P = 0.949$). Only one patient (in the 72 Gy group)

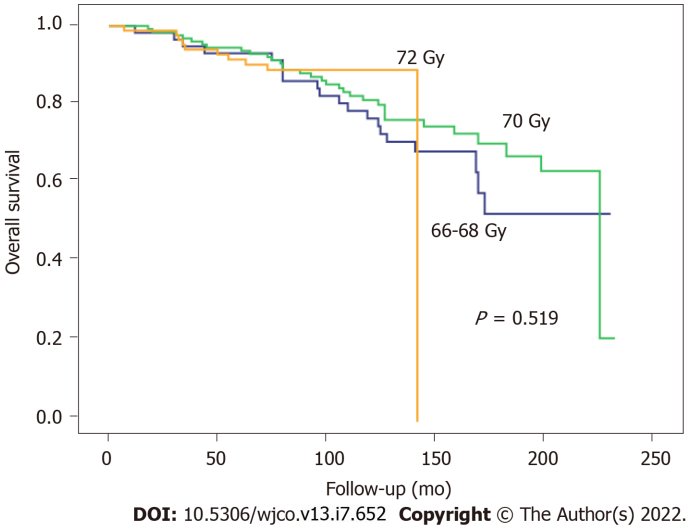


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Figure 3 Overall survival according to total radiotherapy dose to the surgical bed.

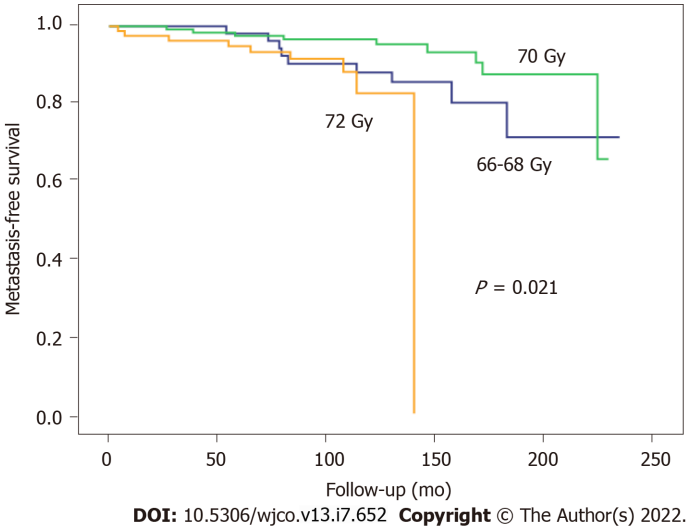

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Figure 4 Metastasis-free survival according to the total radiotherapy dose to surgical bed.

developed G3 toxicity (Table 2). Acute G1 GI toxicity was observed in 16 (29.6%), 23 (26.7%) and 2 (5.6%) patients, respectively, (P = 0.011). Acute G2 toxicity was observed in 2 (3.7%), 6 (6.9%) and 1 (2.8%) patient, (P = 0.278). No cases of acute G3 GI toxicity were observed in any of the groups (Table 2). Chronic GU toxicity was as follows: G1-2 in 3 patients (11.5%) and G3 in one (3.8%) patient in the 70 Gy group, and in 11 (13.4%) and 1 (1.2%) of those who received 72 Gy, respectively (P = 0.338). Chronic G1-G2 GI toxicity was observed in 2 (7.7%) of the patients who received 70 Gy and in one (2%) who received 72 Gy (P = 0.262), with no G3 chronic GI toxicity in any of the groups (Table 3).

DISCUSSION

In this retrospective study, higher total postoperative radiation doses to the surgical bed were not associated with better BFFS or OS outcomes, a finding that is consistent with data from randomised clinical trials[16].

In this series, the patients' clinical characteristics were indicative of an aggressive disease profile: 139 patients (48%) were stage pT3-T4, 47 (16.8%) had a GS ≥ 8, 161 (58.9%) had positive margins in the surgical specimen, and 124 (49%) had a pre-RT PSA > 0.4ng/mL. In addition, 93 patients (34.8%) received short-term androgen blockade prior to surgery, as this was standard clinical practice at some centres based on the available evidence at that time, even though preoperative androgen deprivation therapy is no longer prescribed in these cases[20]. Given the time period (2002-2015) of this study, none of the patients were prescribed concurrent hormonal therapy with postoperative radiotherapy, even

Table 2 Acute gastrointestinal and genitourinary toxicity (Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria) according to total radiotherapy dose to the surgical bed

	Total dose								
	66-68 Gy (*n* = 54)			70 Gy (*n* = 86)			72 Gy (*n* = 36)		
Grade (G)	GI	GII	GIII	GI	GII	GIII	GI	GII	GIII
Acute GU toxicity	11 (20.4%)	2 (3.7%)	0	17 (19.8%)	4 (4.7%)	0	3 (8.3%)	2 (5.6%)	1 (2.8%)
Acute GI toxicity	16 (29.6%)	2 (3.7%)	0	23 (26.7%)	6 (6.9%)	0	2 (5.6%)	1 (2.8%)	0

G: Grade; GI: Gastrointestinal; GU: Genitourinary.

Table 3 Chronic gastrointestinal and genitourinary toxicity (Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria) according to total radiotherapy dose to the surgical bed

	Total dose					
	66-68 Gy (*n* = 5)		70 Gy (*n* = 26)		72 Gy (*n* = 51)	
Grade (G)	GI-II	GIII	GI-II	GIII	GI-II	GIII
Chronic GU toxicity	0	0	3 (11.5%)	1 (3.8%)	11 (13.4%)	1 (1.2%)
Chronic GI toxicity	0	0	2 (7.7%)	0	1 (2%)	0

GI: Gastrointestinal; GU: Genitourinary.

though this approach is now common clinical practice-due to the proven clinical benefits-in well-selected patients who meet the clinical criteria[21,22].

The patients in this series did not undergo prophylactic nodal radiation due to the conflicting and controversial evidence in the literature[23-25]. Nevertheless, only 22 patients (8.3%) developed nodal recurrence; of these, 70% received SRT, 64% presented perineural invasion, and 45% were stage pT3-4. Given the low rate of nodal recurrence in this series, we were unable to identify any significant predictors.

A recent randomised trial compared ART to SRT (64 Gy) without hormonal therapy or prophylactic nodal irradiation[8]. The 5-year BFFS was 87%, which was higher than the 78.8% observed in our series, probably due to the less aggressive disease profile and the application of treatment volumes that differed from those recommended in the Radiation Therapy Oncology Group contouring guidelines[17,18].

In our series, 5- and 10-year BFFS was significantly better in the patients who received ART *vs* SRT, a finding that is consistent with previous reports[3,26-30]. Although numerous studies have sought to determine the optimal timing of EBRT after radical prostatectomy, this remains uncertain[3,27,31,32] Several recent phase III trials-RADICALS, Groupe d´ Etude des Tumeurs Uro-Genitales (GETUG)-AFU 17 and RAVES, and the ARTISTIC meta-analysis-have compared ART to SRT, demonstrating that early SRT is superior to ART[6-9]. However, because those trials included a limited number of patients with highly unfavourable clinicopathologic characteristics (involved margins, pathologic lymph nodes, stage pT3b and/or GS ≥8) more studies are needed to determine whether early SRT is indicated in all surgically-treated patients, or whether some patient subgroups might benefit from ART.

In the comparison of treatment outcomes according to the radiotherapy dose to the surgical bed, dose intensification did not improve the results, a finding that contrasts with several retrospective reports that reported better clinical outcomes in patients who received higher doses[10-15]. However, it is important to emphasize that we did not randomise patients and, moreover, there were important differences among the subgroups in terms of the clinicopathologic characteristics. In fact, this is a study limitation given that patients who received 72 Gy had more severe disease (stage pT3-T4, higher GS) and were less likely to receive hormonal therapy than patients included in the other two subgroups (70 Gy and 66-68 Gy). Both the European Association of Urology (EAU) and the GETUG have developed criteria to identify patients with a high risk of developing metastatic disease in this clinical scenario[21,33]. Nonetheless, in our multivariate analysis, most of these criteria failed to predict the effectiveness of PORT. Consequently, a more comprehensive analysis in a larger sample that stratifies patients according to their baseline clinical characteristics could help to better elucidate the true potential of dose intensification, thus allowing for more individualized treatment.

In terms of toxicity, previous studies have found that dose-escalated PORT is associated with a significant increase in both GU and GI toxicity[16]. However, various factors could influence this

association, including the technique (*i.e.*, 3D-CRT *vs* IMRT), irradiation or not of the uninvolved nodal areas, the contouring criteria for the treatment volumes, pretreatment urinary function, as well as several other factors described elsewhere[34]. We found no significant between-group differences in acute or chronic GI or GU toxicity, regardless of the radiotherapy dose, a finding that is consistent with the randomised trial conducted by Qi *et al*[35]. In that trial, the authors compared outcomes in patients (n = 144) randomised to receive either 66 or 72 Gy to the surgical bed. They found no significant between-group differences in acute and/or chronic GI or GU toxicity. In our study, the use of more advanced radiotherapy techniques (IMRT/rotational techniques) in approximately 50% of the patients may have contributed to the good treatment outcomes. However, these findings should be interpreted cautiously given the retrospective study design and small sample size. Given these limitations, we cannot draw any definitive conclusions. Consequently, larger, more comprehensive studies are needed.

CONCLUSION

The findings of this study suggest that dose-intensified postoperative radiotherapy in patients with PCa is not superior to conventional dosing. Consequently, there is a clear need for randomised clinical trials with well-selected patients to determine the optimal individualized radiotherapy dose scheme in patient subgroups with highly aggressive disease.

ARTICLE HIGHLIGHTS

Research background

Approximately 30% of patients with localized prostate cancer (PCa) who undergo radical prostatectomy will develop biochemical recurrence. In these patients, the only potentially curative treatment is postoperative radiotherapy (PORT) with or without hormone therapy. However, the optimal radiotherapy dose is unknown due to the limited data available.

Research motivation

Our article analyses the changing landscape of the management of prostate cancer patients who receive postoperative radiotherapy, shedding light on an area, optimal radiation dose, applicable to clinical practice, for which the current evidence base is constantly fluctuating with a growing need to optimize the treatment of these patients.

Research objectives

To determine whether the postoperative radiotherapy dose influences biochemical failure-free survival (BFFS) in patients with prostate cancer.

Research methods

Retrospective analysis of patients who underwent radical prostatectomy for PCa followed by PORT-either adjuvant radiotherapy or salvage radiotherapy-between April 2002 and July 2015. From 2002 to 2010, the prescribed radiation dose to the surgical bed was 66-70 Gy in fractions of 2 Gy; from 2010 until the present, the prescribed dose was 70-72 Gy. Patients were grouped into three categories according to the total dose administered: 66-68 Gy, 70 Gy, and 72 Gy. The primary endpoint was BFFS, defined as the post-radiotherapy prostate-specific antigen (PSA) nadir + 0.2 ng/mL. Secondary endpoints were overall survival (OS), cancer-specific survival (CSS), and metastasis-free survival (MFS; based on conventional imaging tests). Treatment-related genitourinary (GU) and gastrointestinal (GI) toxicity was evaluated according to Radiation Therapy Oncology Group/European Organization for Research and Treatment of Cancer criteria. Finally, we aimed to identify potential prognostic factors. BFFS, OS, CSS, and MFS were calculated with the Kaplan-Meier method and the log-rank test. Univariate and multivariate Cox regression models were performed to explore between-group differences in survival outcome measures.

Research results

301 consecutive patients were included. At a median follow-up of 113 mo (range, 4-233), 5-and 10-year BFFS rates were 78.8% and 73.7%, respectively, with OS rates of 93.3% and 81.4%. The 5-year BFFS rates in the three groups were as follows: 69.6% (66-68Gy), 80.5% (70Gy) and 82.6% (72Gy) (P = 0.12): at 10 years, the corresponding rates were 63.9%, 72.9% and 82.6% (P = 0.12), respectively. No significant between-group differences were observed in MFS, CSS, or OS. No significant differences were found in GU or GI toxicity between the 3 radiation-dose groups except acute grade 1 GI toxicity that was observed in 16 (29.6%), 23 (26.7%) and 2 (5.6%) patients in each group (66-68Gy, 70Gy and 72Gy), respectively (P = 0.011).

Research conclusions

Postoperative radiotherapy dose intensification in PCa is not superior to conventional radiotherapy treatment.

Research perspectives

A more comprehensive analysis of the radiation dose in prostate cancer patients who receive postoperative radiotherapy could help to better elucidate the true potential of dose intensification, thus allowing for more individualized treatment.

FOOTNOTES

Author contributions: Hervás A, Domínguez-Rullán JA, Duque Santana V, Valero M, Vallejo C, Sancho S, García Fuentes JD, Cámara Gallego M and López-Campos F contributed to writing, review, editing and supervision; all authors have read and approve the final manuscript.

Institutional review board statement: The study was reviewed and approved by the Ethics Research Committee of Hospital Universitario Ramón y Cajal.

Informed consent statement: Patients were not required to give informed consent to the study because the analysis used anonymous clinical data that were obtained after each patient agreed to treatment by written consent.

Conflict-of-interest statement: The manuscript has been read and approved for submission by all the named authors. All authors declare no conflict of interests for this article.

Data sharing statement: No additional data are available.

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