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A promising therapeutic approach of dendritic cell vaccines for ovarian cancer

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Abstract

Ovarian cancer (OC) remains one of the most lethal gynecological malignancies, primarily due to its often late-stage diagnosis and the development of resistance to conventional therapies. In recent years, significant advancements in immunotherapy have highlighted the potential of dendritic cell (DC) vaccines as a novel therapeutic approach. This review aims to thoroughly evaluate the current landscape and the future potential of DC vaccinations for OC therapy. Recent Studies have provided evidence that DC vaccines can generate specific T-cell responses, thereby enhancing the immunogenicity of ovarian tumors. Furthermore, combining DC vaccines with other therapeutic modalities, such as checkpoint inhibitors and chemotherapy, has shown considerable promise in overcoming the immune evasion mechanisms employed by tumors. However, several challenges remain, including optimizing antigen selection, improving DC maturation and migration, and countering tumor-induced immunosuppression. Continued research is essential for fully unlocking the potential of DC vaccines in improving outcomes for ovarian cancer patients.

Keywords: Ovarian Carcinoma, DC subsets, Immunotherapy, Dendritic Cell Vaccine, Hereditary Ovarian Cancer

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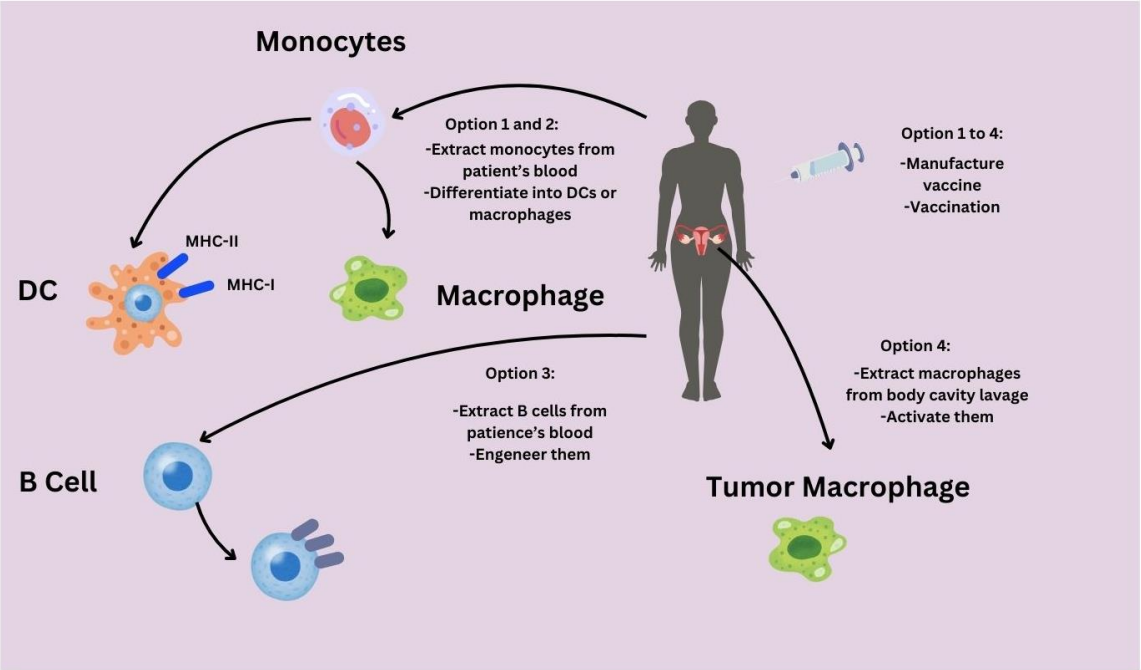
Graphical abstract



Introduction

Ovarian Cancer (OC), a malignant tumor that develops in the ovaries, is often referred to as the "silent killer" due to its subtle symptoms and late diagnosis. It ranks as the seventh leading cause of cancer-related deaths in women and is the deadliest among gynecologic cancers (1, 2). Among female patients, ovarian cancer makes up 4% of all malignancies and 25% of cancers affecting the female reproductive system. It leads to 5% of female deaths and more than 50% of deaths caused by cancer of the female genital tract. The main types of ovarian carcinomas are serous (40%), mucinous (10%), endometrioid carcinoma (20%), undifferentiated carcinoma (10%), and clear cell tumors (3).

Several elements contribute to the prognosis of a tumor, including tumor margin, vascular invasion, tumor grade and stage, expression of oncogenes, and the presence of estrogen and progesterone receptors (3, 4). Immune cells within the tumor, such as Dendritic Cell (DCs), may also serve as a prognostic factor. DCs are a rare immune cell population found in tumors and lymphoid organs, but they play a central role in initiating antigen-specific immunity and tolerance. Manipulating DCs has the potential to effectively induce anti-tumor immunity (5). DCs play a crucial role in the immune system by enhancing immunity or inducing tolerance. This is achieved through the presentation of antigens to T cells, and the delivery of immunomodulatory signals via direct cell-to-cell interactions and the secretion of cytokines (6).

The functions of DCs are influenced by their capacity to sense and respond to environmental stimuli, which are detected through various receptors located on the cell surface and within the cell for cytokines, pathogen-associated molecular patterns (PAMPs), and damage-associated molecular patterns (DAMPs). Recent research underscores the unique functions of DC subsets in antitumor immune responses, offering important insights for therapy and making them a promising tool in vaccine development, especially for diseases like cancer, infectious diseases, and autoimmune disorders (7, 8). To initiate and maintain protective anti-tumor immunity, optimal DC function is necessary. However, aggressive cancers can effectively evade immune control by impairing normal DC functions (9). The understanding of DC subsets and their functions has predominantly been shaped by research in mice; however, there is an increasing interest in exploring the biology of human DCs (10,11). This article will delve into the primary functions of DCs in cancer immunology and examine the potential

therapeutic strategies involving the targeting of DCs in vaccines for patients with OC. Despite all these therapeutic advances, approximately 80–85% of the advanced-stage patients still relapse, indicating the urgent need for novel therapies against OC.

1 .Ovarian Carcinoma

Among women, OC ranks seventh in terms of global cancer diagnosis, following breast, colorectal, lung, endometrial, thyroid, and non-Hodgkin's lymphoma (12). Approximately 239,000 new cases and 152,000 deaths are reported annually (13). Eastern and Central Europe record the highest rates, with 11.4 per 100,000 and 6.0 per 100,000, respectively (6, 13). As a worldwide concern, late diagnosis and the absence of an effective screening strategy contribute to the complexity of the issue. Moreover, newly diagnosed cancer is commonly managed through cytoreductive surgery and platinum-based chemotherapy (14).

Three main cell types - epithelial cells, stromal cells, and germ cells - are responsible for the formation of ovarian tumors, whether they are benign or malignant. In developed nations, more than 90% of malignant tumors are classified as sex cord-stromal tumors. While most epidemiologic research, including this review, emphasizes epithelial OC (15). For instance, granulosa ovarian tumors are derived from epithelial cells. Around 5% to 6% of tumors are cell tumors, like thecomas, whereas germ cell tumors, such as teratomas and dysgerminomas, make up approximately 2% to 3% (13, 16). OC is classified into five distinct histological subtypes, each with identifiable risk factors, cells of origin, molecular compositions, clinical features, and treatments. These subtypes include high-grade serous (HGSOC; 70%), endometrioid (ENOC; 10%), clear cell (CCOC; 10%), mucinous (MOC; 3%), and low-grade serous (LGSOC; <5%) (15) (Figure 1) .

Among these subtypes, high-grade serous carcinoma is the most commonly diagnosed. In contrast, HGSC shares similarities with high-grade endometrioid carcinoma. Among the less frequent histologies, small-cell carcinoma is distinguished by its highly aggressive behavior, often seen in younger women who are diagnosed around the age of 25. The tissue origin of this type of cancer remains uncertain. Additionally, carcinosarcoma, another type of aggressive cancer, is also recognized in certain cases (14, 17). The exact cellular origin and pathogenesis of OC are still unclear. It is interesting to note that a significant proportion of tumors seem to arise from different gynecological tissues, primarily affecting the ovary. Studies on morphology and genetics have shown that the fallopian tube epithelium is the origin of both high- and low-grade serous neoplasms. Furthermore, endometriotic cysts are connected to CCOC and ENOC, while MOC is thought to come from transitional cell nests at the tubal-mesothelial junction. HGSOC and LGSOC are believed to stem from the tubal epithelium, albeit through separate pathways (18).

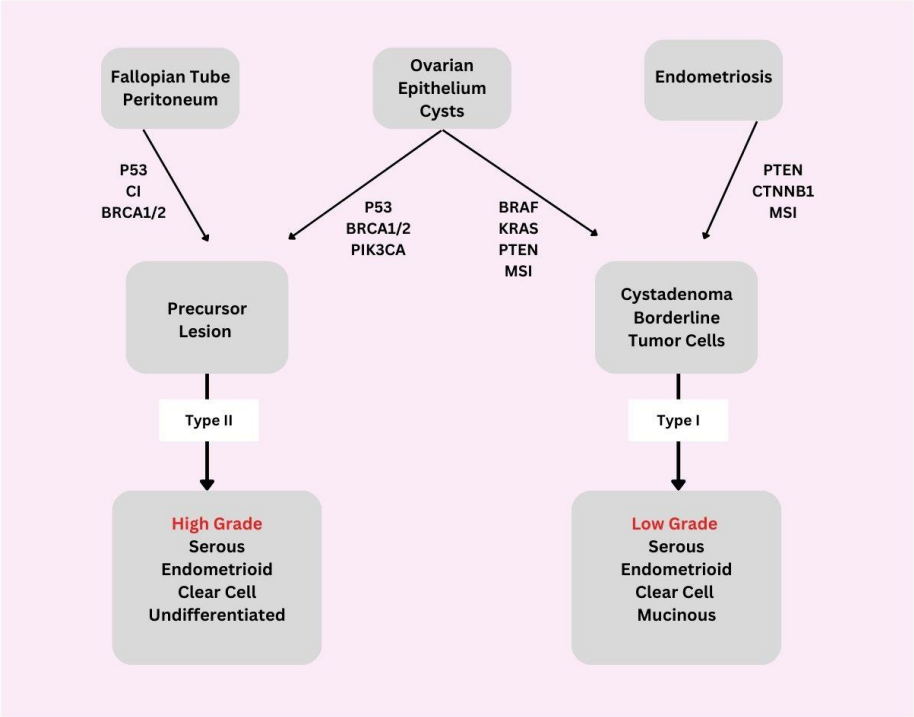


Figure 1. Two-pathway concept of ovarian cancer development (1).

The presence of serous tubal intraepithelial carcinomas, or tubal lesions in the fimbriated end of the fallopian tube, show similarities in morphology and TP53 signatures to tumors. This suggests that the progression of cancer may begin at these tube lesions and advance rapidly into the ovary (2-4,18). LGSOC tumors are identified across a range that signifies a clear progression from benign serous cystadenoma to borderline serous tumors and finally low-grade carcinoma. The glands of epithelial inclusion, believed to have derived from the cystadenoma, are situated in the ovary but display traits similar to those of the fallopian tube, indicating they may have developed from transplanted tubal epithelium (5,16,18). Current epidemiological studies on OC are delving deeper into the investigation of etiologic factors based on histopathologic and molecular subtypes, utilizing the approach of "molecular pathological epidemiology." The evidence from these studies shows that several risk factors have distinct correlations with the primary histotypes (7, 18).

2. Hereditary and Genetic of Ovarian Cancer

Hereditary OC syndromes appear to be genotypically and phenotypically heterogeneous diseases characterized by variable clinical courses (18,19,20). The role of genetic factors in the pathogenesis of OC is well documented. Hereditary OC accounts for at least 5–15% of ovarian carcinomas (18,19). OC risk is influenced by a range of distinct hereditary genetic anomalies (3,21); for example, mutations in the BRCA1 and BRCA2 genes, which are linked to breast cancer, contribute to approximately 90% of OC cases in individuals with a family history of hereditary breast-ovarian cancer. Individuals with BRCA1 mutations have a lifetime risk of OC of approximately 40–50%, while those with BRCA2 mutations have a risk of 20–30% (21). Furthermore, alterations in the BRCA genes elevate the susceptibility to various types of cancer, which include breast cancer, specifically BRCA1 and BRCA2 mutations; pancreatic cancer linked to BRCA2 mutations; prostate cancer associated with BRCA2 mutations; melanoma also connected to BRCA2 mutations; and potentially serous endometrial cancer related to BRCA1 mutations (7,21). Studies have shown that the presence of deleterious mutations in BRCA1/2 and other genes involved in repairing

double-strand DNA breaks is significantly correlated with an increased susceptibility to HGSOC, although these mutations can manifest in other subtypes of tumors as well (21, 22).

Apart from BRCA1 and BRCA2, there are other genetic mutations in genes involved in DNA repair that can raise the chances of developing OC, including genes within the Fanconi anemia-BRCA pathway like RAD51C, RAD51D, BRIP1, BARD1, and PALB2 (22,23). The presence of inherited mutations in other genes involved in DNA repair, namely CHEK2, MRE11A, RAD50, ATM, and TP53, may also contribute to an increased likelihood of OC development (7, 22, 23).

Other inherited disorders, such as Lynch syndrome, are also responsible for an additional 10–15% of hereditary ovarian carcinomas (18,20). The syndrome is characterized by the inheritance of a germline mutation predominantly caused by mutations in four mismatch repair genes (MLH1, MSH2, MSH6, and PMS2), representing 65–85% of cases (23,24). Studies have provided evidence that individuals with Lynch syndrome are more likely to develop endometrioid and clear-cell carcinomas in comparison to the expected occurrence in cases of sporadic OC (7, 25). Despite the involvement of both the BRCA and DNA mismatch repair pathways in DNA repair, the specific reasons behind the occurrence of cancers in particular organs associated with these inherited mutated genes remain understudied (26).

3. Dendritic cells Subsets and Functions in OC

The prognosis of OC is dependent on a variety of factors, including tumor margin, vascular invasion, tumor grade and stage, oncogene expression, and estrogen and progesterone receptor status (9,26). Additionally, the presence of immune cells within the tumor, such as DCs, can serve as an additional prognostic factor (10,27). Considered the most effective antigen-presenting cells, DCs serve as a bridge between the immune system of the host and tumor cells, reflecting their intricate interaction (11,12,27), and despite their limited presence in the body, these cells play a crucial role in triggering antigen-specific immunity and tolerance, making them the predominant cell type (8).

DCs are developed from CD34+ hematopoietic stem cells situated in the bone marrow. Following this, they undergo differentiation into diverse subtypes in the peripheral blood and nonlymphoid organs and tissues, ultimately reaching maturation in the lymphoid organs (13-15). Immature dendritic cells show lower levels of toll-like receptors (TLRs), major histocompatibility complex (MHC) molecules, costimulatory molecules, and adhesion molecules. Consequently, these cells are found in peripheral tissues and have restricted antigen-presenting functions (7, 9, 21).

TLRs are recognized as the key receptors involved in the detection of PAMPs and DAMPs (15,28). Through the activation of DCs, PAMPs stimulate the innate immune response, which serves as a crucial defense against infectious diseases. In the context of tumors, DCs are activated in response to DAMPs released by tumor cells via TLR signaling (12,16,26). Immature DCs respond to chemokine ligands CCL19 and CCL21 by migrating towards the lymph nodes. The maturation of these DCs involves the up-regulation of chemokine receptors CCR7 and CCR8, which enhance their migration (17). While situated in the lymph nodes, they undergo a progressive change into a mature state, marked by an elevated expression of MHC I molecules, MHC II molecules, costimulatory molecules, and adhesion molecules (17,18,28). There are three main subsets into which DCs can be divided: conventional or classical DCs (cDCs, also called myeloid DCs), monocyte-derived DCs (moDCs), and plasmacytoid DCs (pDCs) (8,12,14). cDCs can be further classified as cDC1, cDC2, and migratory DCs (migDCs) (12,13,29) (Figure 2).

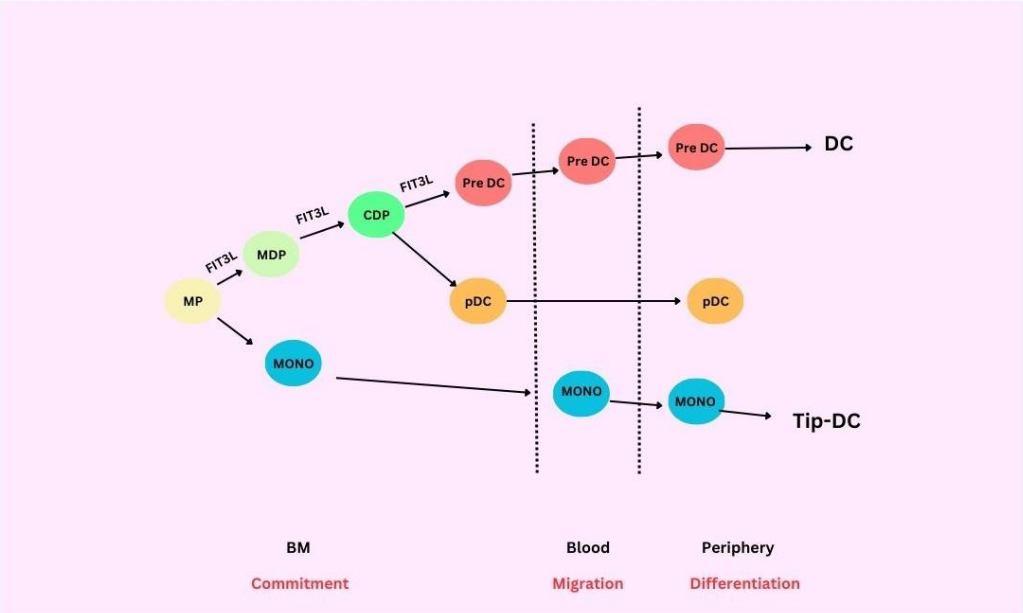


Figure 2. Dendritic cell and monocyte origin and development (29).

3.1 cDC1

cDC1 serves as the primary DC subtype responsible for regulating cancer immunotherapy responses by activating CD8+ T cells via the antigen cross-presentation mechanism (9, 13). They are of utmost importance in facilitating the early activation of CD4+ T cells against tumor-derived antigens via MHC-II, and their role in delivering CD4+ T cell assistance to CD8+ T cells cannot be underestimated (18.29). However, the absence of CDC1s during viral infections disrupts the proper differentiation of memory CD8+ T cells, resulting in unfavorable outcomes (12, 19). cDC1s are also potent in their production of interleukin-12 (IL-12) and have the capability to induce NK and CD8+ T-cell cytotoxicity as well as the generation of interferon-gamma (IFNγ) (19). IFNγ contributes to a positive feedback loop that increases cDC1-mediated IL-12 production, ultimately bolstering antigen cross-presentation (20).

3.2 .cDC2

Classically, cDC2 releases IL-10, IL-12, IL-23, and TNF-b to promote the development of CD4+ helper T cells (9, 13), particularly T helper type 2 (Th2) (18,28) and T helper 17 (Th17) cells (20, 21). These cells are distinct from cDC1s and are unable to functionally fill in for cDC1 deficiencies (12). Studies have indicated that cDC2s can increase the activation of existing CD8+ T cells during anti-CD40 therapy (22). The understanding of cDC2 functions is obstructed by three fundamental hindrances. First, the absence of a definitive marker specific to cDC2 poses a challenge in elucidating the contribution of cDC2s to tissue immune responses in vivo through conditional depletion models. Second, a resemblance can be seen in the present cDC2 markers and phenotypic characteristics with alternative myeloid compartments such as moDCs and macrophages, which poses challenges in isolating the specific contribution of cDC2s in functional inferences compared to other myeloid cells (23,30). Third, the cDC2 compartment is known for its heterogeneity, housing diverse sub-populations. This suggests that each subset within this compartment may possess unique functionalities (10, 24, 25). Various immune contexts have led to the identification and categorization of cDC2 sub-populations, with some overlap in their characteristics. To gain a better understanding, further investigation is necessary. This is especially important for DC vaccines, as targeting the most potent cDC2 subpopulation could potentially improve patient outcomes compared to targeting the entire cDC2 compartment, which may contain some anti-inflammatory sub-populations (23, 29, 30).

3.3. migDC

Migratory DCs, also known as migDCs, DC3, mregDC, or LAMP3+ DCs, are a unique type of fully developed cells that can be found in both cDC1s and cDC2s when they detect or absorb antigens (25, 26). MigDCs are dendritic cells found in non-lymphoid tissues that travel to the tdLN through the lymphatic system instead of the bloodstream. In inflammation, migDCs loaded with antigen move to T-cell regions in LNs to activate CD4+ and CD8+ T cells. They upregulate MHC-II and costimulatory molecules and secrete inflammatory cytokines to enhance T-cell responses (27, 28).

4. Dendritic Cell Dysfunction in The Tumor Microenvironment

Within OC lesions, there is a notable presence of DC infiltration; nevertheless, the infiltrated DCs exhibit a decreased efficacy in antigen presentation owing to DC tolerance. This tolerance is distinguished by the reduced expression of costimulatory molecules on the DC cell surface, leading to a compromised antigen-presenting capability. DCs can assist tumor cells under specific conditions (27, 30). In the absence of tumors, hematopoietic precursors differentiate into progenitors that further specialize into immature DCs. Immature DCs mature and specialize in antigen presentation after meeting an antigen or "danger signal." Nonetheless, differentiation of DCs is commonly disrupted in the tumor microenvironment, resulting in a buildup of defective and immature DCs. In mouse melanoma, tumor-infiltrating DCs contained both myeloid and plasmacytoid DC populations (31). Most of these DCs appeared immature, but about a third expressed a mature phenotype (32).

DC dysfunction can be impacted by immune checkpoint signaling. When PD-1 on T cells interacts with PD-L1 on tumor cells, it can lead to the death of T cells. PD-1 inhibitors could enhance the antitumor effect of DCs in OC (33). Through the release of TGF-b and PGE2 into the microenvironment, OC cells can stimulate the upregulation of PD-L1 in DCs, which strengthens their ability to suppress the immune response of T cells (33,34). Immunosuppressive cells and specific DCs have a direct interaction that affects the body's ability to combat tumors. In ovarian carcinoma, the interaction between pDCs and regulatory T cells (Treg cells) is facilitated by the expression of the ICOS ligand, leading to tumor progression (34). Additionally, insulin-like growth factor (IGF) influences dendritic cells (DCs) in ovarian cancer, impacting cell proliferation, protein synthesis, and growth through the activation of the RAS-ERK and PI3K-AKT pathways. In the presence of IGF, DCs fail to mature and secrete higher levels of IL-10 and TNF-a, considered immunosuppressive factors in the OC microenvironment (35, 36). The insulin-like growth factor type I receptor (IGF1R) is prominent in OC. This receptor has a negative correlation with the differentiation of DCs into cDCs. By utilizing IGF1R inhibitors, the DC-mediated antitumor effect can be rebuilt. This suggests that the IGF axis may be responsible for inducing dysfunction in DCs (36,37). To conclude, immunosuppressive signals contribute to DC dysfunction in OC. By infusing functional DCs into the body, they can engage with T cells in lymph nodes rather than the tumor microenvironment, potentially restoring their ability to present tumor antigens and induce antitumor effects (38, 39).

5. DCs Vaccine in OC

Cancer vaccines are divided into various groups based on how they deliver the chosen TAAs. These groups include cell-based vaccines, peptide/protein vaccines, and genetic vaccines (Table 1) (31, 35).

5.1 cell-based vaccines

Cell-based vaccines can use DCs to help connect innate and adaptive immunity (40). The goal is to trigger cytotoxic T lymphocytes to target and destroy cancer cells using tumor antigens (41,42). DCs are essential for immunosurveillance, which underscores the immune system's vital role in recognizing and removing pathogens and cancer cells. However, the slow progression of malignancy during its initial phases can result in occasional failures of immunosurveillance (39). In the early stages, tumors can occasionally inhibit an immune response or fail to produce the essential signals for immune system activation. Cell-based vaccination aims to fix this problem by reversing the immune system's lack of knowledge about cancer cells (43).

Adjuvant DC vaccines have proven to be effective in the long run for people with melanoma, glioblastoma, prostate cancer, and renal cell carcinoma. However, it

is important to note that these improvements have only been demonstrated in a small number of patients (44, 45). DC vaccination is considered safe and typically causes fewer side effects than chemotherapy and ICBs (39). Choosing the appropriate DC subtypes is a key factor in successful vaccination. The chosen subtypes of autologous DC used in vaccine production display different levels of antigen-presenting potential, potentially influencing the effectiveness of DC vaccines. In the study of DC vaccines for tumors, scientists select particular DC subtypes from peripheral blood cells using apheresis. These subtypes, such as MoDCs, cDCs, and Langerhans cell-type DCs, are assessed in preclinical and clinical studies (36, 45). Various DC subtypes are being targeted to improve immune responses against tumors in vaccines that target DC within and outside the body, and these may vary depending on the cancer types (46,47). To manufacture vaccines that target DCs in the body, there is no need for apheresis to gather autologous DCs. Instead, specific antigens that target receptors on DCs are injected directly into the body. For example, the vaccine CDX-1401 is formulated to target DEC205+ cDC1s in multiple tumors, such as OC. This vaccine includes the DEC205 antibody fused with NY-ESO-1 and a TLR agonist (48, 49). The development of vaccines that target DCs externally involves the use of peripheral blood cells obtained through apheresis (50).

MoDCs are the preferred subtype for this purpose due to the limited number of DCs in peripheral blood cells for vaccine production. On the other hand, a larger number of DCs can be generated from monocytes when cultured in vitro compared to other sources (45). When it comes to vaccinations, cDCs are more potent than MoDCs in inducing long-lasting and broad immune responses. Furthermore, cDCs can enhance the efficacy of immune checkpoint inhibitors (51). The presence of cDC1, cDC2, and pDC in OC has been previously noted. The ratio of cDC and pDC varies in peripheral blood, ascites, and tumor sites. Among DC subsets, pDC is most frequently found in ascites (40) and tumor sites (10), while cDC is more abundant than pDC in the peripheral blood (35). This indicates that peripheral blood could be a valuable resource for the production of DCs (52). Due to the limited number of cDCs available for vaccine manufacturing, MoDCs are commonly used in clinical studies on DC vaccines (27). After isolation from peripheral blood using apheresis, mononuclear cells are cultured in vitro with GM-CSF and IL-4 for a specific duration. The evaluation of markers on DCs, including CD11c+, HLA-DR+, HLA-ABC+, CD40+, CD80+, CD83+, CD86+, and CCR7+, is performed to monitor the cellular composition of the DC vaccine (53). However, these markers are not effective in distinguishing MoDCs from other DC subtypes, resulting in the DC vaccine being a combination of DCs and a small proportion of other peripheral blood cells (11, 27) (Figure 3).

5.2 Peptide/Protein-Based Vaccines

Autologous cancer vaccines, such as DCs or whole tumor cells, are limited by the need for patient samples and the complex process of making personalized vaccines. Recombinant vaccines have an advantage in this respect. Peptide- or protein-based vaccines typically utilize specific TAAs and are given with an adjuvant or immune modulator to enhance uptake by DCs (3,53). Many different peptides have been experimented within OC to find out if they can target HER-2/neu. HER-2/neu is a member of the HER/EGFR/ERBB family, and if it's amplified in breast cancer, it makes the cancer more aggressive. That's why it's an important target for around 20%–30% of patients (54). The presence of HER-2/neu overexpression or amplification has been detected in OC cases (19), suggesting it as a potential target for cancer vaccination. Nevertheless, studies using HER-2/neu peptides have not shown any immune response (14, 36), and there is no clinical data available (31). The most efficient outcomes in OC treatment through peptide-based vaccines have been achieved by employing a personalized peptide vaccine (PPV). This method consists of mixing four peptides (selected from a set of 31) that have been tested for immune response in every patient and then injecting them subcutaneously in Montanide ISA51VG (19, 31, 35). The study revealed that platinum-sensitive patients had a median survival time of 39.2 months, while platinum-resistant patients had 16.2 months. Standard of care patients had 18–30 months (platinum-sensitive) and 8–12 months (platinum-resistant). Notably, PPV not only enhanced immune responses to specific peptides but also extended to other peptides, resulting in longer survival (50). The findings indicated that selecting and administering vaccine antigens based on the patient's

pre-existing immunity before vaccination could extend overall survival in advanced OC patients (55).

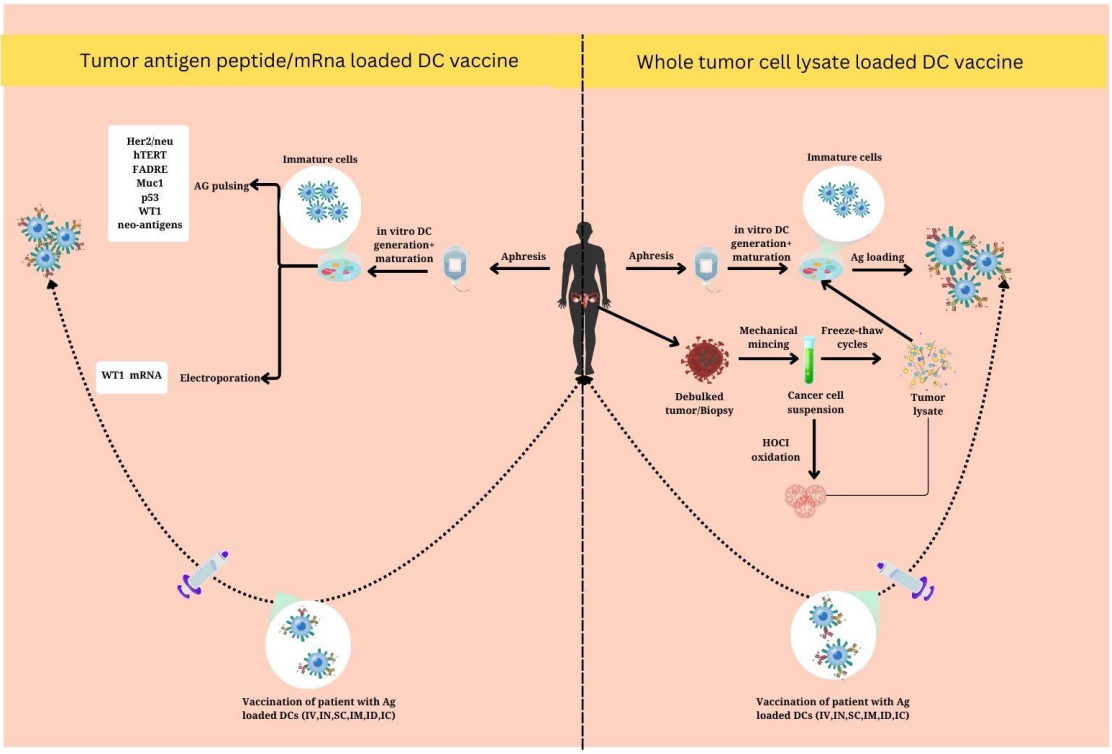


Figure 3. An overview of dendritic cell vaccination strategies used in ovarian carcinoma. Ag, antigen; HOCl, hypochlorous acid; IV, intravenous; IN, intranodal; SC, subcutaneous; ID, intradermal; IC, intracutaneous.

5.3 Genetic Vaccine

The use of genetic vaccines, whether they are DNA, RNA, or virus-based, can activate the expression of chosen TAAs within somatic cells like keratinocytes, myocytes, or DCs that infiltrate muscle or skin at the vaccination site. This can result in either cross-priming or direct antigen presentation to infiltrating T-cells. Genetic vaccines make it easy to deliver multiple antigens in one immunization, activate different branches of immunity, and have a more cost-effective and standardized manufacturing process (30). Two viral vaccines have been tested for OC: One team is concentrating on the "cancer-testis" antigen NY-ESO-1, which has been integrated into vaccinia (rV) as the initial vaccine and fowlpox (rF) as the follow-up vaccine. The second genetic vaccine tested for ovarian cancer, PANVAC-C + PANVAC-V, is a Poxviral vaccine. It involves engineering CEA-MUC1-TRICOM (B7.1, ICAM-1, LFA-3) into vaccinia (PANVAC-V) as the prime and fowlpox (PANVAC-C) as the booster vaccination (37, 38). A Phase I clinical trial with 25 patients with CEA- or MUC1-expressing metastatic cancers, including three with OC, showed limited clinical activity. Ongoing studies are investigating different genetic vaccines for treatment (56,57,58).

Table 1. Published results from therapeutic vaccines tested in ovarian cancer from 2000 to 2024.

Class	Name	Description	Clinical Development Phase	No. of Pts (OvCa Pts)	Clinical Result	Ref
DCs	APCEDEN	DCs loaded with whole-tumor lysate	Phase II	38 pts (9 OvCa pts)	No CR observed; ORR was 28.9% (11/38) and irRC was 42.1%	**(35)**
	DCVax-L	DCs loaded with autologous oxidized tumor lysate, combined with bevacizumab and metronomic Cy	Pilot	6 OvCa pts	4/6 pts (66%) achieved clinical benefit (including 2 PR and 2 SD)	**(37)**
	OCDC	DCs loaded with autologous oxidized tumor lysate	Pilot	5 OvCa pts	2/5 pts (40%) demonstrated PFS2 > PFS1	**(30)**
	DC-MFP	DCs loaded with mannan-MUC1 fusion protein (MFP)	Phase I	9 pts (2 OvCa pts)	2/9 pts (22%) in progression at entry were stable after therapy, for at least 3 years	**(33)**
	DC-wtl	DCs loaded with crude whole tumor lysate	Phase I	8 pts (6 OvCa pts)	Data suggested a positive correlation with disease stabilization	**(33)**
	Lapuleucel-T, Neuvenge, APC 8024	DCs loaded with BA7072, a fusion protein HER-2/neu linked to GM-CSF	Phase I; HER-2+ tumors	18 pts (4 OvCa pts)	2/18 pts (11%) had SD lasting > 48 weeks	**(24)**
	HER-2/neu; MUC1 peptides	DCs loaded with synthetic peptides derived from HER-2/neu or MUC1 peptides	Phase I; HER-2+ or MUC1+ tumors	10 pts (3 OvCa pts), HLA-A*02+	No data	**(24)**
	hTERT; HER-2/neu; PADRE peptides	DCs loaded with synthetic peptides derived from hTERT; HER-2/neu; PADRE	Phase I/II	14 OvCa pts, HLA-A*02+	3 years-OS was 90%; 3 years-PFS was 80% (with Cy)	**(24)**
	WT-1; MUC1; CA125	DCs loaded with syntheticpeptides derived from WT-1; MUC1; CA125	Phase II	56 OvCa pts	DCR and ORR were 29% and 3.6%, respectively	**(35)**
Peptides/ proteins	Mixture of peptides (comparison)	Predesigned peptides vs. PPV (personalized peptide vaccine); admixed with Montanide ISA-51	Pilot	14 pts (5 OvCa pts), HLA-A*02+ or HLA-A*24+	No clinical response with predesigned; 3/5 cervical cancer pts (60%) showed objective	**(41)**

				tumor regression	
Mixture of different peptides	OvCa-associated peptides plus a helper peptide from tetanus toxoid protein, admixed with Montanide ISA-51 and GM-CSF	Phase I	9 OvCa pts, HLA-A*01+, -A*02+ or A*03+	One participant remained disease-free at 19 months after active treatment	**(41)**
Mixture OvCa-associated peptides	OvCa-associated peptides admixed with Montanide ISA-51 and GM-CSF	Pilot	15 pts (8 OvCa pts); HLA-A*02+	With median follow-up of 492 days, 4 OvCa pts had relapsed and 3 died (expected relapse rate 18–22 mo in 75% of pts)	**(42)**
HER-2/neu	Epitope p369–377, admixed with GM-CSF	Phase I; HER-2/neu++ Tu	6 pts (2 OvCa pts), HLA-A*02+	No data	**(42)**
HER-2/neu-ICD	ICD protein, aas 676–1255, His-tagged	Phase I; HER-2/neu++ Tu	29 pts (1 OvCa pt)	No data	**(45)**
NY-ESO-1	Epitope p157–170, admixed with Montanide ISA-51	Phase I	18 OvCa pts, HLA-DPB1 *0401+ or *0402+	Median PFS of 19.0 mo (vs. 16–18 weeks in pts receiving 2nd line chemo)	**(46)**
NY-ESO-1 OLP	NY-ESO-1 overlapping long peptides, +/´ Montanide and Poly-ICLC	Phase I	28 OvCa pts (HLA indep)	Pts NY-ESO-1+ receiving OLP + Montanide + Poly-ICLC showed delayed time to recurrence	**(57)**
NY-ESO-1 protein	NY-ESO-1 protein + Montanide + CM-CSF +/´ decitabine	Phase I	12 OvCa pts	5/10 (50%) pts had SD (median duration 6.3 mo), and 1/10 (10%)had PR (duration 5.8 mo)	**(57)**
P53	Wt p53: 264–272 peptide admixed with GM-CSF and Montanide ISA-51, either SC (Arm A) or loaded into DCs (Arm B)	Phase II; p53++ Tu;	21 OvCa pts, HLA-A*02:01+	No significant difference between arms in median OS (40.8 mo vs. 29.6 mo, p = 0.26), nor in PFS (4.2 mo vs. 8.7 mo, p = 0.94)	**(68)**
P53-SLP	Ten synthetic peptides 25–30 aa long overlapping peptides (aas 70–248 in	Phase II	18 OvCa pts	2/18 (11%) of pts with SD, not clearly	**(35)**

		wt-p53) admixed in Montanide ISA-51		(HLA indep)	attributable to vaccination	
	Flt3-L	Truncated glycoprotein Flt3-L (Fms-like tyr kinase-3-ligand, which increases DCs and monocytes), either i.p. or s.c.	Pilot	15 pts (9 OvCa pts)	No objective responses were observed	**(35)**
	PPV	Personalized peptide vaccine: mixture of 4 peptides (from a panel of 31) previously tested for immunity in each pt, admixed in Montanide ISA51VG	Phase II	42 OvCa pts (HLA-dep)	Median survival time (MST) was 39.2 mo in platinum-sensitive pts, vs. 16.2 mo in platinum-resistant	**(58)**
Whole tumor cells	Fang vaccine, Vigil™ Ovarian, Gemogenovatucel-T	Autologous tumor cells eletroporated with FANG vector, a plasmid encoding GM-CSF and a bi-shRNA targeting furin convertase, thereby downregulating TGF-b1 and b2	Phase I	27 pts (5 OvCa pts)	23/26 pts (88%) showed SD at month 2 or later	**(32)**
Genetic vaccines	PANVAC-C + PANVAC-V	Poxviral vaccine: CEA-MUC1-TRICOM (B7.1, ICAM-1, LFA-3) engineered into vaccinia (PANVAC-V) as prime and fowlpox (PANVAC-C) as booster vaccination	Pilot; CEA+ or MUC1+ Tu	25 pts (3 OvCa pts)	1 OvCa pt (1/25: 4%) had durable (18 mo) clinical response	**(39)**
	rV-NY-ESO-1 + rF-NY-ESO-1	NY-ESO-1 engineered into vaccinia (rV) as prime and fowlpox (rF) as booster vaccination	Phase I; NY-ESO-1+ Tu	36 pts (1 OvCa pt)	7/9 pts with stage II/IV MEL survived 17–63+ mo	**(39)**

Abbreviations: aas, aminoacids; CR, complete response; DCR, disease control rate (SD + PR + CR); irRC, immune-related response criteria; mo, months; MST, median survival time; ORR, objective response rate (PR + CR); OS, overall survival; PD, progressive disease; PFS, progression free survival; PR, partial response; Pt(s), patient(s); SD, stable disease; TTP, time to progression.

6. DCs in the cancer therapy

DCs have the potential to influence the efficacy of cancer therapies currently employed in clinical practice. This review delves into the impact that DCs can have on the response to such treatments (7).

6.1 Chemotherapy and DCs

Traditionally, chemotherapeutic treatments such asbortezomib, doxorubicin, epirubicin, idarubicin, and Mitoxantrone and oxaliplatin have long been thought to provide anti-cancer benefits by either directly killing cancer cells or causing a permanent cessation of the cell cycle, and these responses depend on DCs (16,59). It was believed that chemotherapy could target rapidly dividing cells, including immune cells, and cause immunosuppression. Many chemotherapy drugs used in clinics are not immunogenic or have immunosuppressive side effects. They can directly inhibit or kill effector cells or indirectly cause energy or immune paralysis. As a result, the immune system's role in anticancer therapy has been largely ignored (18). It is now commonly believed that certain chemotherapy drugs and anticancer medications can trigger the body's immune system to fight tumors (19, 60).

One way they do this is by making tumor cells more visible to the immune system, which leads to an immune response against the tumor. This has been shown in experiments with mice that have a healthy immune system. Additionally, immunogenic cell death (ICD) may be induced by specific physical methods like UV-C irradiation, hypericin-based photodynamic therapy, and high hydrostatic pressure, while certain oncolytic viruses possess the intrinsic capacity to initiate ICD. These were among the chemotherapeutic drugs used in clinical practice: anthracyclines (doxorubicin, epirubicin, and idarubicin), mitoxantrone, oxaliplatin, CTX, and bleomycin (BLM) (29, 60). The efficacy of these stimulants in triggering an immune response against tumors relies on the development of adaptive stress reactions that facilitate the synchronized release of endogenous danger signals from apoptotic cells. These signaling molecules, referred to as DAMPs, interact with various receptors found on dendritic cells to activate the adaptive branch of the immune system (61). Multiple DAMPs have been identified as characteristic elements of ICD, specifically the initial presentation of the endoplasmic reticulum (ER) chaperone calreticulin (CRT) and heat-shock proteins (HSPs) HSP70 and HSP90; the spontaneous release of molecules like high mobility group box 1 (HMGB1); and the excretion of adenosine triphosphate (ATP) (10, 31, 62). In addition, some chemotherapy drugs can induce tumor cells to produce type I interferons (IFNs). Although type I IFNs are not DAMPs specifically, they have strong immune-boosting effects and are crucial for chemotherapy-induced cell death to be recognized as immunogenic. To conclude, the activation of the immune system is supported by DAMPs, as demonstrated in many in vitro tumor cell line models and in vivo mouse immunization experiments. Recent reports also suggest that monitoring DAMPs in cancer patients may have prognostic or predictive value (30, 32).

6.2 Radiation therapy and DCs

Highly proliferating cells are the preferred targets of radiation treatment. While this therapy's primary function is to directly kill cancer cells, this explanation falls short of explaining the therapy's overall effect on tumor growth. Radiation therapy's anti-tumor efficacy also involves local bystander effects, such as the release of DAMPs and cytotoxic mediators, the alteration of the immunological TME, and the in situ generation of reactive oxygen species (63, 64). Additionally, radiation therapy can generate distant effects, referred to as out-of-field or abscopal effects, that are correlated with the promotion of systemic immune responses against cancer, facilitated by the induction of immunogenic cell death and the activation of CD8+ T cells by cDC1 Following radiation therapy, cancer cells release cytosolic DNA that acts as a DAMP, signaling through cGAS-STING to stimulate type I interferon production by DCs, thus aiding in antitumor immunity (55). However, high radiation doses prompt the expression of DNase TREX1, which breaks down cytosolic DNA, limiting interferon production and the immunostimulatory impact on cDC1s (54).

6.3 Small-molecule inhibitors and DCs

Small-molecule inhibitors have been developed to target important oncogenic signaling pathways such as STAT3 and mitogen-activated protein β-catenin signaling (26). These pathways are associated with decreased cDC1 tumor infiltration and a lack of response to immune checkpoint blockade therapy. Nevertheless, the transfer of preactivated in vitro-generated cDC1-like cells with poly(I:C)5 was effective in reversing this non-responsiveness (8). Moreover, the combination of vaccination with naturally existing cDC1s loaded with immunogenic cell death-derived whole tumor antigen and anti-PD1 treatment reveals a synergistic outcome. The synergy between TLR-induced activation of DCs and ICB can be heightened by FLT3L-induced expansion of DC populations. Recent discoveries suggest that cDC1 is vital for cross-priming, as evidenced by WDFY4-deficient mice being incapable of rejecting immunogenic tumors due to a defect in a vesicular transport pathway necessary for cross-presentation (18, 32). Enhancing the function of DCs may result in improved and expanded responsiveness to ICB regimens. Both cGAS and STING are crucial for intrinsic antitumor immunity and effective responses to anti-PDL1, with DCs playing a key role in mediating these responses. (33). The activation of type I interferons to stimulate cDC1s can potentially improve the response to anti-PDL1 treatment, indicating a potential requirement for the activation of tumor DCs to support effector T cell activity triggered by ICB.

Enhancing the production of chemokines like CXCL9 and CXCL10 by DCs, possibly through epigenetic modifications, may also enhance the efficacy of ICB therapy (32, 34).

7. Safety of Dendritic Cell Vaccines

The safety of DC vaccines has generated significant interest due to their potential to modify immune cell, cytokine, and chemokine levels in the body. Thankfully, most OC patients involved in clinical studies have responded well to DC vaccines. Most reported side effects are grade 1 or 2 and include common symptoms like local skin reactions, fatigue, pain, flu-like symptoms, muscle aches, fever, nausea, and vomiting (32). Numerous studies have reported serious toxicity associated with DC vaccines, especially when used in combination with other treatments. During the phase II trial of a p53 peptide cancer vaccine and DC vaccine, every one of the 21 patients encountered a localized skin response. Among the participants who were administered a combination DC vaccine containing p53 peptide, a minimum of 3 patients documented lymphopenia and fatigue (32). Additional toxicities related to the grade III/IV vaccine consist of increased ALT and AST levels, fever, hypocalcemia, memory impairment, and rigors (53). It is important to highlight that notable toxicity was connected to the IL-2 treatment in the subgroup examination of this research. This was noted during a phase I clinical trial of the DC vaccine for the maintenance therapy of ovarian carcinoma (39, 40). Additionally, two patients suffered from hypertension. More evidence is necessary to determine if these toxicities are related to DC vaccines in OC patients undergoing chemotherapy. To conclude, DC vaccines are usually well tolerated, but combining them with chemotherapy or immunotherapy should be done carefully (Table 2) (23, 65,66).

Table 2. Issues and challenges in cancer vaccine development (35).

Issues	Challenges
Personalised vaccination (e.g., patient tumour/ immune cells)	A. Development of a robust and standardisable vaccination platform technology Poor/undetectable immune response B. Generation of a strong immune response against tumour antigens without inducing unwanted auto-immune reactions
Immune tolerance and tumour escape	A. Counteract mechanisms of immune evasion by cancer B. Absence of efficacy biomarkers C. Establishment of immune surrogates of anti-tumour efficacy
Immunotherapy as single agent	A. Development of rational combination therapies B. Efficacy driven by tumour shrinkage endpoint C. Design clinical trials that incorporate new concepts of immune-related response criteria
Self-limited immunity	A. Maintenance of anti-tumor immune response over time

8. Future of the DC Vaccines

DC vaccines have exhibited promise in the realm of immunotherapy for ovarian carcinoma. However, there exists untapped potential that necessitates exploration through the use of new technologies, cohort studies, and biomarkers. Tumor immunosuppressive signals have been found to impair dendritic cells, leading to compromised immunological function and metabolism, thereby resulting in issues related to antigen presentation and tumor growth (40). The rise in popularity of personalized DC vaccines can be attributed to their effectiveness in activating T cell responses that target tumor antigens specific to individual patients, facilitated by next-generation sequencing and bioinformatics analysis. Nonetheless, challenges such as complex preparation techniques, limited tumor samples, and difficulties in selecting tumor antigens need to be addressed. While clinical experiments have validated the safety of DC vaccines,

their efficacy varies depending on the manufacturing technique and study strategy. The identification of an ideal biomarker is essential in this context (41).

Conclusion

Advances in cancer immunotherapy, notably for ovarian carcinoma, have demonstrated their significance in the battle against cancer. Cykine therapy, peptide vaccines, monoclonal antibodies, dendritic cell-based vaccines, adoptive T cell transfer, immune checkpoint inhibitors, and various nanoparticles are all being studied for ovarian cancer treatment. Combining these tactics with individual therapy can help boost the immunological response. However, there is still potential to enhance treatment options, such as by studying tumor biology, immune-suppressive networks, and immunomodulatory techniques. Polymeric and lipid-based nanoparticles are being created to deliver antigens, immune stimulants, and immunoadjuvants in a sustained-release manner. More research is needed to create accurate biomarkers and successful treatment combinations.

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References

1. Huarte, E., et al., Depletion of dendritic cells delays ovarian cancer progression by boosting antitumor immunity. Cancer research, 2008. 68(18): p. 7684-7691.

2. Scarlett, U.K., et al., Ovarian cancer progression is controlled by phenotypic changes in dendritic cells. Journal of Experimental Medicine, 2012. 209(3): p. 495-506.

3. Martin-Lluesma, S., et al., Are dendritic cells the most appropriate therapeutic vaccine for patients with ovarian cancer? Current opinion in biotechnology, 2020. 65: p. 190-196.

4. Chae, C.-S., E. Teran-Cabanillas, and J.R. Cubillos-Ruiz, Dendritic cell rehab: new strategies to unleash therapeutic immunity in ovarian cancer. Cancer Immunology, Immunotherapy, 2017. 66: p. 969-977.

5. Aarnio, M., et al., Cancer risk in mutation carriers of DNA-mismatch-repair genes. International journal of cancer, 1999. 81(2): p. 214-218.

6. Chen, W., et al., Cancer statistics in China, 2015. CA: a cancer journal for clinicians, 2016. 66(2): p. 115-132.

7. Ricciardelli, C. and M.K. Oehler, Diverse molecular pathways in ovarian cancer and their clinical significance. Maturitas, 2009. 62(3): p. 270-275.

8. Wculek, S.K., et al., Dendritic cells in cancer immunology and immunotherapy. Nature Reviews Immunology, 2020. 20(1): p. 7-24.

9. Del Prete, A., et al., Functional role of dendritic cell subsets in cancer progression and clinical implications. International journal of molecular sciences, 2020. 21(11): p. 3930.

10. Merad, M., et al., The dendritic cell lineage: ontogeny and function of dendritic cells and their subsets in the steady state and the inflamed setting. Annual review of immunology, 2013. 31(1): p. 563-604.

11. Plantinga, M., et al., Conventional and monocyte-derived CD11b+ dendritic cells initiate and

maintain T helper 2 cell-mediated immunity to house dust mite allergen. Immunity, 2013. 38(2): p. 322-335.

12. Kossaï, M., et al., Ovarian cancer: a heterogeneous disease. Pathobiology, 2018. 85(1-2): p. 41-49.

13. Reid, B.M., J.B. Permuth, and T.A. Sellers, Epidemiology of ovarian cancer: a review. Cancer biology & medicine, 2017. 14(1): p. 9.

14. Matulonis, U.A., et al., Ovarian cancer. Nature reviews Disease primers, 2016. 2(1): p. 1-22.

15. McCluggage, W.G., Morphological subtypes of ovarian carcinoma: a review with emphasis on new developments and pathogenesis. Pathology-Journal of the RCPA, 2011. 43(5): p. 420-432.

16. Sankaranarayanan, R. and J. Ferlay, Worldwide burden of gynaecological cancer: the size of the problem. Best practice & research Clinical obstetrics & gynaecology, 2006. 20(2): p. 207-225.

17. Witkowski, L., et al., The influence of clinical and genetic factors on patient outcome in small cell carcinoma of the ovary, hypercalcemic type. Gynecologic oncology, 2016. 141(3): p. 454-460.

18. Veras, E., et al., Cystic and adenofibromatous clear cell carcinomas of the ovary: distinctive tumors that differ in their pathogenesis and behavior: a clinicopathologic analysis of 122 cases. The American journal of surgical pathology, 2009. 33(6): p. 844-853.

19. Kindelberger, D.W., et al., Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: evidence for a causal relationship. The American journal of surgical pathology, 2007. 31(2): p. 161-169.

20. Li, J., et al., Tubal origin of 'ovarian'low-grade serous carcinoma. Modern Pathology, 2011. 24(11): p. 1488-1499.

21. Gerhard, G.M., et al., Tumor-infiltrating dendritic cell states are conserved across solid human cancers. Journal of Experimental Medicine, 2021. 218(1).

22. Lynch, H.T., et al., Hereditary ovarian carcinoma: heterogeneity, molecular genetics, pathology, and management. Molecular oncology, 2009. 3(2): p. 97-137.

23. Ford, D., et al., Genetic heterogeneity and penetrance analysis of the BRCA1 and BRCA2 genes in breast cancer families. The American Journal of Human Genetics, 1998. 62(3): p. 676-689.

24. Beesley, J., et al., Association between single-nucleotide polymorphisms in hormone metabolism and DNA repair genes and epithelial ovarian cancer: results from two Australian studies and an additional validation set. Cancer Epidemiology Biomarkers & Prevention, 2007. 16(12): p. 2557-2565.

25. Reid, S.D., G. Penna, and L. Adorini, The control of T cell responses by dendritic cell subsets. Current opinion in immunology, 2000. 12(1): p. 114-121.

26. Lynch, H.T., C. Bewtra, and J.F. Lynch, Familial peritoneal ovarian carcinomatosis: a new clinical entity? Medical Hypotheses, 1986. 21(2): p. 171-177.

27. Zhang, X., et al., Dendritic cell vaccines in ovarian cancer. Frontiers in Immunology, 2021. 11: p. 613773.

28. Eisenthal, A., et al., Expression of dendritic cells in ovarian tumors correlates with clinical outcome in patients with ovarian cancer. Human pathology, 2001. 32(8): p. 803-807.

29. Caro, A.A., et al., Dendritic cell vaccines: A promising approach in the fight against ovarian cancer. Cancers, 2022. 14(16): p. 4037.

30. Fitzgerald, K.A. and J.C. Kagan, Toll-like receptors and the control of immunity. Cell, 2020. 180(6): p. 1044-1066.

31. Ozkan, E. and F. Bakar-Ates, The trinity of matrix metalloproteinases, inflammation, and cancer: A literature review of recent updates. Anti-Inflammatory & Anti-Allergy Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents), 2020. 19(3): p. 206-221.

32. Qu, C., et al., Role of CCR8 and other chemokine pathways in the migration of monocyte-derived dendritic cells to lymph nodes. The Journal of experimental medicine, 2004. 200(10): p. 1231-1241.

33. Sarivalasis, A., et al., Cell therapies in ovarian cancer. Therapeutic Advances in Medical Oncology, 2021. 13: p. 17588359211008399.

34. Murgaski, A., et al., Efficacy of CD40 agonists is mediated by distinct cDC subsets and subverted by suppressive macrophages. Cancer Research, 2022. 82(20): p. 3785-3801.

35. Guilliams, M., et al., Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. Nature Reviews Immunology, 2014. 14(8): p. 571-578.

36. Collin, M. and V. Bigley, Human dendritic cell subsets: an update. Immunology, 2018. 154(1): p. 3-20.

37. Maier, B., et al., A conserved dendritic-cell regulatory program limits antitumour immunity. Nature, 2020. 580(7802): p. 257-262.

38. Mueller, S.N., Spreading the load: Antigen transfer between migratory and lymph node-resident dendritic cells promotes T-cell priming. European Journal of Immunology, 2017. 47(10): p. 1798-1801.

39. Van Willigen, W.W., et al., Dendritic cell cancer therapy: vaccinating the right patient at the right time. Frontiers in Immunology, 2018. 9: p. 2265.

40. Liu, K. and M.C. Nussenzweig, Origin and development of dendritic cells. Immunological reviews, 2010. 234(1): p. 45-54.

41. Ma, Y., et al., Dendritic cells in the cancer microenvironment. Journal of Cancer, 2013. 4(1): p. 36.

42. Stoitzner, P., et al., Inefficient presentation of tumor-derived antigen by tumor-infiltrating dendritic cells. Cancer Immunology, Immunotherapy, 2008. 57: p. 1665-1673.

43. Flies, D.B., et al., Immune checkpoint blockade reveals the stimulatory capacity of tumor-associated CD103+ dendritic cells in late-stage ovarian cancer. Oncoimmunology, 2016. 5(8): p. e1185583.

44. Conrad, C., et al., Plasmacytoid dendritic cells promote immunosuppression in ovarian cancer via ICOS costimulation of Foxp3+ T-regulatory cells. Cancer research, 2012. 72(20): p. 5240-5249.

45. Anguille, S., et al., Clinical use of dendritic cells for cancer therapy. The lancet oncology, 2014. 15(7): p. e257-e267.

46. Huang, C.-T., et al., Insulin-like growth factors inhibit dendritic cell-mediated anti-tumor immunity through regulating ERK1/2 phosphorylation and p38 dephosphorylation. Cancer letters, 2015. 359(1): p. 117-126.

47. Somri-Gannam, L., et al., IGF1R axis inhibition restores dendritic cell antitumor response in ovarian cancer. Translational Oncology, 2020. 13(8): p. 100790.

48. Goyne, H.E. and M.J. Cannon, Dendritic cell vaccination, immune regulation, and clinical outcomes in ovarian cancer. Frontiers in immunology, 2013. 4: p. 382.

49. Tanyi, J.L., et al., Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. Science translational medicine, 2018. 10(436): p. eaao5931.

50. Odunsi, K., et al., Vaccination with an NY-ESO-1 peptide of HLA class I/II specificities induces integrated humoral and T cell responses in ovarian cancer. Proceedings of the National Academy of Sciences, 2007. 104(31): p. 12837-12842.

51. Aurisicchio, L. and G. Ciliberto, Genetic cancer vaccines: current status and perspectives. Expert opinion on biological therapy, 2012. 12(8): p. 1043-1058.

52. Truxova, I., et al., Rationale for the combination of dendritic cell-based vaccination approaches with chemotherapy agents. International Review of Cell and Molecular Biology, 2017. 330: p. 115-156.

53. Duwa, R., J.-H. Jeong, and S. Yook, Immunotherapeutic strategies for the treatment of ovarian cancer: current status and future direction. Journal of Industrial and Engineering Chemistry, 2021. 94: p. 62-77.

54. An, D., S. Banerjee, and J.-M. Lee, Recent advancements of antiangiogenic combination therapies in ovarian cancer. Cancer treatment reviews, 2021. 98: p. 102224.

55. Deng, L., et al., STING-dependent cytosolic DNA sensing promotes radiation-induced type I interferon-dependent antitumor immunity in immunogenic tumors. Immunity, 2014. 41(5): p. 843-852.

56. Mastelic-Gavillet, B., et al., Quantitative and qualitative impairments in dendritic cell subsets of

patients with ovarian or prostate cancer. European Journal of Cancer, 2020. 135: p. 173-182.

57. Guo, C., et al., Therapeutic cancer vaccines: past, present, and future. Advances in cancer research, 2013. 119: p. 421-475.

58. Wirth, T.C., J.T. Harty, and V.P. Badovinac, Modulating numbers and phenotype of CD8+ T cells in secondary immune responses. European journal of immunology, 2010. 40(7): p. 1916-1926.

59. Fucikova, J., et al., Induction of tolerance and immunity by dendritic cells: mechanisms and clinical applications. Frontiers in immunology, 2019. 10: p. 2393.

60. Dhodapkar, M.V., et al., Induction of antigen-specific immunity with a vaccine targeting NY-ESO-1 to the dendritic cell receptor DEC-205. Science translational medicine, 2014. 6(232): p. 232ra51-232ra51.

61. Zhou, Y., et al., Vaccine efficacy against primary and metastatic cancer with in vitro-generated CD103+ conventional dendritic cells. Journal for immunotherapy of cancer, 2020. 8(1).

62. Vazquez, J., et al., Identification of unique clusters of T, dendritic, and innate lymphoid cells in the peritoneal fluid of ovarian cancer patients. American Journal of Reproductive Immunology, 2020. 84(3): p. e13284.

63. Zhang, W., et al., Phase I/II clinical trial of a Wilms' tumor 1-targeted dendritic cell vaccination-based immunotherapy in patients with advanced cancer. Cancer Immunology, Immunotherapy, 2019. 68: p. 121-130.

64. Mitri, Z., T. Constantine, and R. O′ Regan, The HER2 receptor in breast cancer: pathophysiology, clinical use, and new advances in therapy. Chemotherapy research and practice, 2012. 2012(1): p. 743193.

65. Gulley, J.L., et al., A pilot study to evaluate the safety and clinical outcomes of vaccination with recombinant CEA-MUC-1-TRICOM (PANVAC) poxviral-based vaccines in patients with metastatic carcinoma. Clinical cancer research: an official journal of the American Association for Cancer Research, 2008. 14 (10): p. 3060.

66. Fard, A.A. and A. Mahmoodzadeh, Unraveling the Progression of Colon Cancer Pathogenesis Through Epigenetic Alterations and Genetic Pathways. Cureus, 2024. 16(5).

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Prevalence and clinical significance of saprophytic bacteria in bloodstream infections among cancer patients

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Abstract

Introduction: Bloodstream infections (BSIs) in cancer patients are associated with high morbidity and mortality. While common pathogens are well-studied, the role of saprophytic bacteria in BSIs among this population is less understood. To investigate the prevalence and clinical significance of saprophytic pathogens causing BSIs in cancer patients at a tertiary care center.

Materials and Methods: This retrospective study included all 200 consecutive adult cancer patients with suspected sepsis over four months. Blood cultures were processed on an automated BACTEC system. Subculture and identification were performed using standard microbiological techniques and the Vitek 2 system. Antimicrobial sensitivity was performed as per CLSI guidelines.

Results: The blood culture positivity in these patients was 79% (158/200). Of the 158 positive blood cultures, 10.1% (16/158) were saprophytic pathogens. These included *Enterococcus avium, Sphingomonas paucimobilis, Actinomyces meyeri, Kodamaea ohmeri, Elizabethkingia meningoseptica, Aeromonas hydrophila, Achromobacter xylosoxidans, Stenotrophomonas maltophilia, Pantoea dispersa,* and *Burkholderia pseudomallei*. The overall 30-day mortality rate for patients with saprophytic pathogen BSIs was 20%.

Conclusion: Saprophytic bacteria have gained recognition as possible human pathogens, especially in immunocompromised patients including cancer patients. Such high-risk patients should be put on empiric antibiotics to improve patient outcomes till the time clinical significance is established.

Keywords: Bloodstream infection, Saprophytic organism, Cancer patients

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Introduction

Bloodstream infections (BSIs) remain a significant cause of morbidity and mortality in cancer patients, with mortality rates ranging from 18% to 42% (1-3). It has been known for decades that the fundamental cause of the life-threatening organ damage seen in sepsis is not the direct result of the invading organisms but rather the host response to infection(1,2). Additionally, patients who survive sepsis endure long-term physical, psychological, and cognitive impairment, known as post-sepsis syndrome (3,4).

Blood culture remains the gold standard for diagnosing BSI. While common pathogens like *Klebsiella pneumoniae* and *Escherichia coli* are well-recognized in this setting, the role of saprophytic bacteria in causing BSIs among cancer patients is less understood(5-7).

The immunocompromised state of cancer patients, coupled with frequent hospitalizations and invasive procedures, creates a unique environment where typically non-pathogenic organisms can cause severe infections(8-12). A recurring challenge in clinical practice is distinguishing true pathogens from colonizers and contaminants in blood cultures. This study aimed to investigate the prevalence and clinical significance of saprophytic pathogens causing BSIs in cancer patients in a large tertiary care center

Material and methods

This retrospective study was carried out over four months, from January to April 2023 at one of the large tertiary care referral center. A total of 200 consecutive patients (age ≥ 18 years) with a confirmed diagnosis of cancer presenting with signs and symptoms of bloodstream infection were included in the study. Non-cancer patients or cancer patients with polymicrobial bloodstream infections or where the clinical significance of the isolate could not be determined were excluded from the study.

As a routine hospital protocol, venous blood was taken aseptically from patients clinically suspected of having bloodstream infections. The blood was inoculated aseptically into the automated blood culture bottle and incubated using the BACTEC system. Once flagged positive, the blood culture bottles (PBC) were processed using standard microbiological techniques. Briefly, direct gram staining was done from PBC along with subculture on blood agar and MacConkey agar. The plates were incubated at 37°C, and the next day growth was observed. The colonies were identified by colony characteristics, gram stain, and biochemical reactions. Identification was confirmed by the Vitek 2 system (Biomerieux, France). Antimicrobial susceptibility testing was carried out by the Vitex 2 system as well as manually using the disk diffusion method and the antibiotics tested were chosen either from the CLSI guidelines or the available literature where CLSI guidelines was not available.

Statistical Analysis: Descriptive statistics were used to summarize patient demographics and clinical characteristics. Categorical variables were presented as frequencies and percentages. Continuous variables were expressed as means and ranges. Fisher's exact test was used to compare mortality rates between groups. A p-value <0.05 was considered statistically significant. All analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY).

Data Collection and Analysis: Clinical data including patient demographics, cancer type, presenting symptoms, and treatment outcomes were collected from medical records. The frequency of saprophytic pathogens was calculated as a percentage of total isolates.

The data for this study were collected as part of routine clinical care and were fully anonymized. It is essential to highlight that all patient data were de-identified to maintain confidentiality. Personal identifiers were removed prior to data analysis, and no identifiable information was used in the study. This approach ensured compliance with patient privacy regulations and ethical standards. There was no ethical consideration regarding the study.

Results

Out of 200 patients, 60 (30%) were female and 140 (70%) were male. The mean age was 52 years (range: 18-75 years). The most common cancer types were colorectal (25%), lung (20%), and hematological malignancies (15%) as shown in Figure 1.

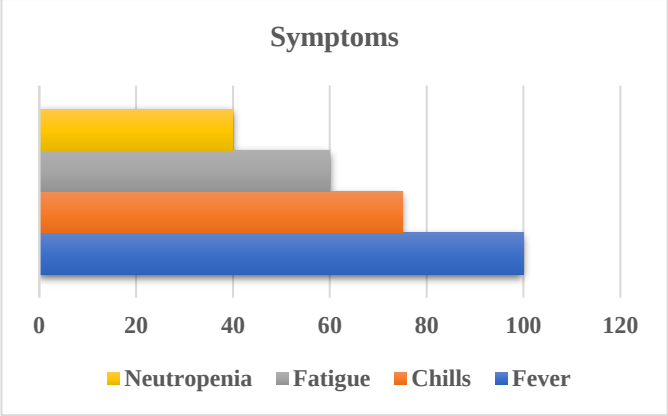

Figure 1. Symptoms prevalence.

A total of 158 organisms were isolated from 200 patients, indicating a culture positivity rate of 79%. Common pathogens such as *Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli, Enterococcus spp, and Staphylococcus aureus accounted for 142 (89.9%) of isolates, while 16 (10.1%) isolates were identified as saprophytic pathogens. This included Enterococcus avium, Sphingomonas paucimobilis, Actinomyces meyeri, Kodamaea ohmeri, Elizabethkingia meningoseptica, Aeromonas hydrophila, Achromobacter xylosoxidans, Stenotrophomonas maltophilia, Pantoea dispersa, Burkholderia pseudomallei* as shown in table 1.

The most common presenting symptoms were fever (100%), chills (75%), and fatigue (60%). Neutropenia was present in 40% of cases. The overall 30-day mortality rate for patients with saprophytic pathogen BSIs was 20%, compared to 18% for those with common pathogens (p=0.42, Fisher's exact test) (Figure 2).

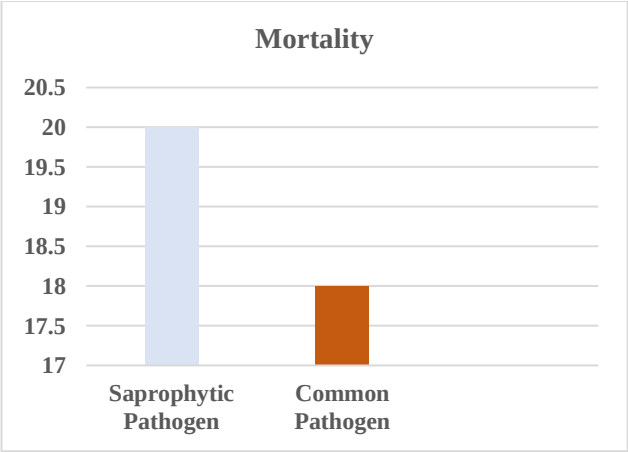

Figure 2. Mortality Rate.

Table 1. Demographic and Clinical Characteristics of Patients with Saprophytic Pathogen BSIs.

Pathogen	Number of Cases	Mortality Rate (%)
Enterococcus avium	1	0
Sphingomonas paucimobilis	2	50
Actinomyces meyeri	1	0
Kodamaea ohmeri	1	100
Elizabethkingia meningoseptica	2	0
Aeromonas hydrophila	2	0
Achromobacter xylosoxidans	2	0
Stenotrophomonas maltophilia	2	0
Pantoea dispersa	1	0
Burkholderia pseudomallei	2	50

Enterococcus avium (7-12)
A 60-year-old male with metastatic colon cancer on chemotherapy presented with fever, lethargy, and unresponsiveness. Blood tests showed leukopenia and thrombocytopenia with elevated lactate levels. Blood cultures revealed *Enterococcus avium*, sensitive to ampicillin, vancomycin, and linezolid, but resistant to high-level gentamicin, ciprofloxacin, levofloxacin, and erythromycin. Vancomycin was administered, resulting in clinical improvement and resolution of fever over 7 days. *Enterococcus avium* is a rare pathogen in humans, often found in birds, and requires prompt diagnosis and treatment, especially in immunocompromised patients.

Sphingomonas paucimobilis (13-17)
Case 1: A 37-year-old male with metastatic pancreatic cancer had a fever and chills. *Sphingomonas paucimobilis*, sensitive to ciprofloxacin, ceftazidime, ceftriaxone, meropenem, and imipenem, was isolated from blood cultures. Treatment with ceftriaxone led to clinical improvement and sterile follow-up cultures.

Case 2: A 20-year-old male with meningioma presented with fever and headache. Blood culture grew *Sphingomonas paucimobilis*, sensitive to ceftazidime and ceftriaxone but resistant to ciprofloxacin, meropenem, and imipenem. The patient succumbed to septicemia despite ceftriaxone treatment.

Actinomyces meyeri (18-19)
A 46-year-old woman with cervical cancer on chemotherapy presented with fever and hypotension. Blood culture initially showed no growth but later identified *Actinomyces meyeri.* Sensitive to penicillin, ciprofloxacin, and amoxicillin-clavulanic acid, she was treated with penicillin but left the hospital against medical advice after 3 days of worsening condition. *Actinomyces meyeri* is a rare pathogen, typically part of polymicrobial infections, and is often underdiagnosed.

Kodamaea ohmeri (20-22)
A 28-year-old male with colorectal adenocarcinoma and traumatic pancreatic injury presented with abdominal distention, poor appetite, and weight loss. Kodamaea ohmeri, sensitive to amphotericin B, itraconazole, and voriconazole but resistant to fluconazole, was isolated. Despite voriconazole therapy, the patient's condition deteriorated rapidly, leading to death. *Kodamaea ohmeri* is an emerging opportunistic pathogen with high mortality rates.

Elizabethkingia meningoseptica (23-24)
Case 1: A 58-year-old male with meningioma had a fever and weakness. Elizabethkingia meningoseptica, sensitive to ciprofloxacin, amikacin, and minocycline, was isolated. Ciprofloxacin treatment led to significant clinical improvement.

Case 2: A 63-year-old male with metastatic lung cancer had respiratory symptoms and fever. Blood culture grew *Elizabethkingia meningoseptica*, sensitive to ciprofloxacin and resistant to gentamicin. Ciprofloxacin treatment resolved symptoms and follow-up cultures were negative. *Elizabethkingia meningoseptica* is an emerging nosocomial pathogen often associated with high mortality in cancer patients.

Aeromonas hydrophila (25-26)
Case 1: A 62-year-old male with chronic lymphoid leukemia presented with fever and dizziness. *Aeromonas hydrophila*, sensitive to multiple antibiotics, was treated with meropenem, leading to symptom resolution.

Case 2: An HIV-positive patient with colorectal cancer and a recent leg injury presented with fever and elevated leukocytes. *Aeromonas hydrophila*, sensitive to multiple antibiotics including trimethoprim-sulfamethoxazole, was treated successfully. *Aeromonas hydrophila* is increasingly recognized as a significant pathogen in immunocompromised patients.

Achromobacter xylosoxidans (27-28)
Case 1: A 64-year-old male with colon cancer and a 58-year-old female with pancreatic cancer, both with type 2 diabetes, presented with fever and chills. *Achromobacter xylosoxidans*, sensitive to ciprofloxacin, was isolated. Both patients responded well to ciprofloxacin treatment. *Achromobacter xylosoxidans* can cause significant infections, particularly in immunocompromised individuals.

Stenotrophomonas maltophilia (29-30)
Case 1: A 67-year-old male with sigmoid adenocarcinoma had persistent fever and cough. *Stenotrophomonas maltophilia*, treated with trimethoprim-sulfamethoxazole and levofloxacin, showed clinical improvement.

Case 2: A 60-year-old male with glioblastoma presented with fever and altered mental status. *Stenotrophomonas maltophilia* was treated with trimethoprim-sulfamethoxazole and ceftazidime with gradual improvement. *Stenotrophomonas maltophilia* is challenging to diagnose and manage but responds well to trimethoprim-sulfamethoxazole.

Pantoea dispersa (31)
A 35-year-old chronic alcoholic with liver cirrhosis presented with abdominal pain, fever, and vomiting. *Pantoea dispersa*, sensitive to minocycline, was treated effectively, leading to the resolution of symptoms. *Pantoea dispersa*, while less virulent, can cause significant infections in immunocompromised individuals.

Burkholderia pseudomallei (32-34)
Case 1: A 57-year-old male with colon cancer improved significantly with imipenem therapy after isolation of *Burkholderia pseudomallei.*

Case 2: A 43-year-old female with pulmonary tuberculosis and ovarian cancer succumbed to septic shock despite aggressive treatment. *Burkholderia pseudomallei*, endemic to tropical regions, poses a high mortality risk and highlights the need for early detection and preventive measures.

This study shows that saprophytic pathogens account for a notable proportion of bloodstream infections in cancer patients, emphasizing the need for accurate identification and targeted treatment, particularly for high-mortality organisms like *Kodamaea ohmeri and Burkholderia pseudomallei*.

Discussion

Our study reveals that saprophytic pathogens account for a significant proportion (10.1%) of bloodstream infections (BSIs) in cancer patients, highlighting the importance of considering these organisms in the differential diagnosis of BSIs, especially in immunocompromised hosts. This finding is consistent with recent literature that has increasingly recognized the role of opportunistic pathogens in causing severe infections in vulnerable populations (1,2).

The prevalence of saprophytic pathogens in our study (10.1%) is slightly higher than that reported by Rega et al (6), who found a 7.5% prevalence of unusual bacterial isolates in BSIs among Ethiopian cancer patients (3). This difference might be attributed to variations in geographical location, patient population, or improvements in diagnostic techniques. Our findings underscore the need for clinicians to maintain a high index of suspicion for atypical pathogens in cancer patients presenting with signs of BSI.

Of particular note was the isolation of *Kodamaea ohmeri* and *Burkholderia pseudomallei*, both associated with high mortality rates. *K. ohmeri*, once considered a benign organism, has emerged as an opportunistic pathogen capable of causing invasive infections in immunocompromised individuals (4). Similarly, *B. pseudomallei*, the causative agent of melioidosis, is increasingly recognized as a significant threat to immunocompromised patients, particularly in endemic regions (5). These findings align with recent global surveillance data that highlight the growing importance of emerging pathogens in healthcare-associated infections (6).

Our statistical analysis revealed no significant difference in 30-day mortality rates between patients with saprophytic pathogen BSIs and those with common pathogens (20% vs. 18%, p=0.42). This finding is intriguing and contrasts with some previous studies that have reported higher mortality rates associated with unusual pathogens (7,8).

The successful treatment of some cases with targeted antimicrobial therapy in our study demonstrates the importance of prompt and accurate identification of these pathogens. This observation is supported by recent literature emphasizing the critical role of rapid diagnostics and appropriate antimicrobial stewardship in managing BSIs, particularly those caused by unusual pathogens (9,10)

Our study also highlights the challenges in distinguishing true pathogens from colonizers or contaminants, particularly in the case of saprophytic organisms. This dilemma is well-recognized in clinical microbiology and emphasizes the need for careful interpretation of blood culture results in the context of the patient's clinical presentation (13,14). The implementation of clinical decision support systems and machine learning algorithms shows promise in aiding clinicians in this complex decision-making process (15).

The high rate of neutropenia (40%) observed in our cohort of cancer patients with BSIs is consistent with previous studies and underscores the vulnerability of this population to opportunistic infections (16,17). Recent research has focused on strategies to prevent and manage infections in neutropenic cancer patients, including the use of prophylactic antimicrobials and immunomodulatory agents (18,19). Our findings support the need for tailored approaches to infection prevention and management in this high-risk group.

While our study provides valuable insights into the prevalence and clinical significance of saprophytic pathogens in cancer-associated BSIs, it has several limitations. As a single-center study with a relatively small sample size, particularly for saprophytic pathogen infections, the statistical power of our comparisons is limited. This may have prevented us from detecting significant differences in outcomes between groups. Additionally, the short duration of the

study precluded analysis of seasonal variations in pathogen distribution and potential confounding factors that may influence patient outcomes. These limitations highlight the need for larger, multi-center studies with longer follow-up periods to more comprehensively characterize the epidemiology and clinical impact of saprophytic pathogen BSIs in cancer patients.

Despite these limitations, our study contributes to the growing body of evidence highlighting the importance of saprophytic pathogens in BSIs among cancer patients. The findings underscore the need for heightened awareness among clinicians, improved diagnostic strategies, and tailored antimicrobial approaches for managing these infections. Future research should focus on developing rapid diagnostic tools specifically targeted at identifying unusual pathogens, as well as exploring novel therapeutic strategies for managing infections caused by these emerging organisms**.**

Conclusion

Our study demonstrates that saprophytic pathogens account for a significant proportion (10.1%) of BSIs in cancer patients, with no statistically significant difference in mortality rates compared to common pathogens(1-6,25). These findings underscore the importance of considering these organisms in the differential diagnosis of BSIs, especially in immunocompromised hosts. Further large-scale, multicenter studies are needed to better understand the epidemiology and clinical impact of saprophytic pathogen BSIs in cancer patients.

In the present study, the patients were started on early empiric therapy and almost 75% of the patients responded to the treatment. The response to treatment in the present study reiterates that the presence of saprophytic bacteria from cases of BSI should not be ignored in cancer patients. It would be worthwhile to start the patient on early empiric treatment till the time a repeat blood culture is sent for confirmation of the clinical significance of these isolates. Raising awareness among healthcare providers about the potential for such infections is crucial to ensure timely diagnosis and intervention.

Author contribution

ShG: Conceptualization, Data curation, Formal analysis, Methodology, Software, Validation, Visualization, Writing original draft, Writing review & editing. **WW:** Writing review & editing, Resources, Software, Data curation, Methodology, Project administration, Software, Validation. **MJ:** Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing original draft, Writing review & editing. **PL:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources. **Ash:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision. **ShT:** Investigation, administration.

Conflict of interest

The authors declare that they have no competing interests.

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Ethical approval

This is a retrospective study and there is no ethical consideration related to paper. The data for this study was collected as part of routine clinical care and was fully anonymized. All patient data were de-identified to maintain confidentiality. Personal identifiers were removed prior to data analysis, and no identifiable information was used in the study. This approach ensures compliance with patient privacy regulations and ethical standards.

References

1. Mu S, Xiang H, Wang Y, et al. The pathogens of secondary infection in septic patients share a similar genotype to those that predominate in the gut. Crit Care. 2022;26:68.

2. McCreery RJ, Florescu DF, Kalil AC. Sepsis in Immunocompromised Patients Without Human Immunodeficiency Virus. J Infect Dis. 2020;221(2):156–65.

3. Van der Slikke EC, An AY, Hancock REW, Bouma HR. Exploring the pathophysiology of post-sepsis syndrome to identify therapeutic opportunities. EBioMedicine. 2020;61:103044.

4. Singer M, Deutschman MC. Improving the prevention, diagnosis and clinical management of sepsis. WHO. 2017 Jan 9.

5. Viscoli C. Bloodstream Infections: The peak of the iceberg. Virulence. 2016;7(3):248–51.

6. Rega B, Wolde-Amanuel Y, Adane K, Belay E, Abubeker A, Asrat D, et al. Saprophytic bacterial isolates causing bloodstream infections in Ethiopian patients with cancer. Infect Agent Cancer. 2017;12:40.

7. Patel R, Keating MR, Cockerill III FR, Steckelberg JM. Bacteremia Due to Enterococcus avium. Clin Infect Dis. 1993;17(6):1006–11.

8. Bhagat B, Shah T, Roistacher K, Glatt AE. Enterococcus avium infection. Infect Dis Clin Pract1996;5(8):502–3.

9. Mirzoyev Z, Anavekar N, Wilson F, Uslan D, Baddour L, Mookadam F. Enterococcus avium endocarditis. Scand J Infect Dis. 2004;36(11–12):876–8.

10. Osoba AO, Kutub H, Waliuddin A. Enterococcus avium: An unusual cause of cerebral abscess. Neurosci J. 2005;10(4):297–300.

11. Shin BS, Jang NY, Park HE, Cheung YD, Park HS. A Case of Enterococcus Avium Colitis with Endoscopic Finding. Gastroenterol Hepatol. 2017;8(1):00267.

12. Abo-Zed A, Hegazy S, Phan T. Detection of Enterococcus avium in a case of urinary tract infection and haematuria. Access Microbiol. 2022;4(5):000349.

13. Laupland KB, Paterson DL, Stewart AG, Edwards F, Harris PN. Sphingomonas paucimobilis bloodstream infection. Int J Infect Dis. 2022 Jun;119:172-177.

14. Tito E, Ahmad A, Gongolli J, Issack W, Johnson A. Sphingomonas paucimobilis Bacteremia in a Patient with Retropharyngeal Abscess. Cureus. 2022;14(5).

15. Nandy S, Dudeja M, Das AK, Tiwari R. Community Acquired Bacteremia by Sphingomonas paucimobilis: Two Saprophytic Case Reports. J Clin Diagn Res. 2013;7(12):2947–9.

16. Cheong HS, Wi YM, Moon SY, Kang CI, Son JS, Ko KS, et al. Clinical features and treatment outcomes of infections caused by Sphingomonas paucimobilis. Infect Control Hosp Epidemiol. 2008;29(10):990–2.

17. Lin JN, Lai CH, Chen YH, Lin HL, Huang CK, Chen WF, et al. Sphingomonas paucimobilis bacteremia in humans: 16 case reports and a literature review. J Microbiol Immunol Infect. 2010;43(1):35–42.

18. Apothéloz C, Regamey C. Disseminated infection due to Actinomyces meyeri: case report and review. Clin Infect Dis. 1996;22(4):621–5.

19. Verrienti G, Megliola G, Antonaci E, Gisotti A, Raccagni C. Actinomyces meyeri Causing Cerebral Abscess in a Patient on Methotrexate: A Saprophytic Case Report and Systematic Review of the Literature. Cureus. 2023;15(6):e41204.

20. Diallo K, Lefevre B, Cadelis G, Gallois JC, Gandon F, Nicolas M. A case report of fungemia due to Kodamaea ohmeri. BMC Infect Dis. 2019;19(1):1-3.

21. Zhou M, Li Y, Kudinha T, Xu Y, Liu Z. Kodamaea ohmeri as an Emerging Human Pathogen: A Review and Update. Front Microbiol. 2021;12:736582.

22. Zhou M, Yu S, Kudinha T, Xiao M, Wang H, Xu Y, et al. Identification and antifungal susceptibility profiles of Kodamaea ohmeri based on a seven-year multicenter surveillance study. Infect Drug Resist. 2019;12:1657–64.

23. Li Y, Liu T, Shi C, et al. Epidemiological, clinical, and laboratory features of patients infected with Elizabethkingia meningoseptica at a tertiary hospital in Hefei City, China. Front Public Health. 2022;10:964046.

24. Jean SS, Lee WS, Chen FL, Ou TY, Hsueh PR. Elizabethkingia meningoseptica: an important emerging pathogen causing healthcare-associated infections. J Hosp Infect. 2014;86(4):244–9.

25. Hughes WT, Armstrong D, Bodey GP, et al. Guidelines for the Use of Antimicrobial Agents in Neutropenic Patients with Cancer. Clin Infect Dis. 2002;34(6):730–51.

26. Okumura K, Shoji F, Yoshida M, Mizuta A, Makino I, Higashi H. Severe sepsis caused by Aeromonas hydrophila in a patient using tocilizumab: a case report. J Med Case Rep. 2011;5(1):1-3.

27. Isler B, Kidd TJ, Stewart AG, Harris P, Paterson DL. Achromobacter infections and treatment options. Antimicrob Agents Chemother. 2020;64(11):10-128.

28. Swenson CE, Sadikot RT. Achromobacter respiratory infections. Ann Am Thorac Soc. 2015;12(2):252-8.

29. Said MS, Tirthani E, Lesho E. Stenotrophomonas Maltophilia. [Updated 2023 Jun 12]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 Jan. Available from: https://www.ncbi.nlm.nih.gov/books/NBK572123/

30. Cho SY, Lee DG, Choi SM, Park C, Chun HS, Park YJ, et al. Stenotrophomonas maltophilia bloodstream infection in patients with hematologic malignancies: a retrospective study and in vitro activities of antimicrobial combinations. BMC Infect Dis. 2015;15:1-8.

31. Asai N, Koizumi Y, Yamada A, Sakanashi D, Watanabe H, Kato H, et al. Pantoea dispersa bacteremia in an immunocompetent patient: a case report and review of the literature. J Med Case Rep. 2019;13(1):1-5.

32. Barman P, Sidhwa H, Shirkhande PA. Melioidosis: A Case Report. J Glob Infect Dis. 2011;3(2):183–6.

33. Meumann EM, Cheng AC, Ward L, Currie BJ. Clinical features and epidemiology of melioidosis pneumonia: results from a 21-year study and review of the literature. Clin Infect Dis. 2012;54(3):362-9.

34. Hussin A, Nor Rahim MY, Dalusim F, Shahidan MA, Nathan S, Ibrahim N. Improving the clinical recognition, prognosis, and treatment of melioidosis through epidemiology and clinical findings: The Sabah perspective. PLoS Negl Trop Dis. 2023;17(10):e0011696.

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Platelet count/spleen diameter ratio for the non-invasive diagnosis of esophageal varices in Iranian patients with cirrhosis

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Abstract

Introduction: Esophageal varices (EVs) carry a significant risk of rupture and subsequent life-threatening bleeding. While previous research has investigated the effectiveness of the platelet count to spleen diameter ratio (PC/SD) as a non-invasive predictor of EVs in various populations, this study specifically focuses on the Iranian population to assess the applicability and effectiveness of this parameter in this region.

Materials and Methods: Upper gastrointestinal endoscopy was performed on 147 cirrhotic patients to screen for EVs. Biochemical tests and ultrasonography were done to measure spleen diameter (SD) and calculate the PC/SD ratio. ROC analysis was done to determine the predictive performance of the parameters.

Results: Among the patients, 73% had EVs. The analysis showed the following: platelet count (PC) had an AUC of 0.695 with 78.7% sensitivity and 56.4% specificity; SD had an AUC of 0.750 with 49.1% sensitivity and 89.7% specificity; and the PC/SD ratio had an AUC of 0.734 with 60.2% sensitivity and 79.5% specificity. The PC/SD ratio exhibited a high positive predictive value of 93% but a low negative predictive value of 41.9%. Optimal cutoff values were determined as follows: PC≤ 100,000, SD< 163, and PC/SD ratio≤ 523.

Conclusion: By identifying high-risk patients who may benefit from targeted endoscopic screening, this non-invasive method could contribute to improving overall patient care and reducing the need for invasive procedures. However, due to suboptimal performance results, it is crucial to use this approach with caution, as endoscopic screening remains the standard practice for the diagnosis and management of esophageal varices.

Keywords: Platelet count, Spleen diameter, Platelet count/spleen diameter ratio, Hepatic cirrhosis, Esophageal varices

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Introduction

Portal hypertension, a consequence of chronic liver disease and cirrhosis, represents a significant clinical challenge, with the development of esophageal varices (EV) being one of its most serious complications (1, 2). EVs are abnormally dilated veins in the lower portion of the esophagus, and their rupture can lead to life-threatening variceal bleeding, a primary factor contributing to morbidity and death in cirrhotic patients (3, 4).

Epidemiological studies have reported that the prevalence of EVs in cirrhotic patients can range from 60% to 80%, depending on the underlying etiology and severity of the liver disease. Furthermore, among patients with established EVs, the annual risk of experiencing a first episode of bleeding is estimated to be 10% to 15%, with a mortality rate as high as 20% associated with this event (5).

The gold standard for diagnosing EVs is upper endoscopy, which enables direct inspection and grading of the varices (6). However, this invasive procedure requires specialized equipment and skilled personnel, potentially limiting its accessibility and cost-effectiveness, especially in resource-constrained healthcare settings (5, 7).

In order to address these issues, scientists have looked into the use of non-invasive techniques to determine high-risk cirrhotic patients who would most benefit from targeted endoscopic screening. One such approach is the Platelet Count/Spleen Diameter (PC/SD) ratio, which has been proposed as a reliable predictor of the presence and severity of EVs (7-10). This simple, cost-effective, and easily obtainable parameter has the potential to optimize resource allocation and improve access to necessary care for cirrhotic patients.

However, it is important to note that the performance of predictive models, such as the PC/SD ratio, may vary across different populations due to factors like underlying disease etiology, genetic differences, and environmental influences (10). Therefore, it is crucial to assess the clinical utility of these non-invasive diagnostic tools in particular populations, such as the Iranian population in this study, to ensure their validity and clinical utility.

In light of the aforementioned situation, the current investigation was carried out to explore the correlation between platelet count (PC), spleen diameter (SD), and their ratio (PC/SD) in cirrhotic patients within the Iranian population. By focusing on this unique group, we seek to determine how effectively the PC/SD ratio functions in different healthcare settings and geographic regions. Our goal is to establish a non-invasive, cost-effective tool that can enhance early detection and improve patient management strategies in local healthcare environments.

Methods

Study Design and Population

This analytical cross-sectional study was conducted at Razi Hospital, a tertiary care center in the north of Iran, Rasht city. The study population comprised cirrhotic patients referred to the gastroenterology department at the study site between September 15, 2023, and March 15, 2024. A combination of clinical, laboratory, and imaging results led to the diagnosis of cirrhosis in the patients and all adult individuals with a confirmed diagnosis of cirrhosis, regardless of the underlying etiology, were included. However, patients diagnosed with acute liver failure, those requiring urgent liver transplantation, pregnant women, and individuals unable or unwilling to comply with study procedures were excluded from this study.

Data Collection

A thorough clinical evaluation that included a physical examination, a medical history, and laboratory testing was performed on each recruited individual. The age, sex, and marital status of the participants were documented. As part of the study protocol, the PC was measured for each participant and reported in the unit of × 10^9/L. All participants underwent abdominal ultrasonography, performed by an expert radiologist. The bipolar diameter of the spleen was precisely measured and recorded in millimeters (mm). Also, an experienced gastroenterologist performed upper endoscopy. The presence of EVs was meticulously assessed and documented. The ratio of PC to SD was computed by dividing the PC (× 10^9/L) by the SD (mm) measured during the abdominal ultrasonography.

Statistical Analysis

When applicable, the mean ± standard deviation or median (interquartile range) were used to express continuous variables. Frequencies and percentages were used to display the categorical variables. To assess the normality of the key variables (PC, SD, and PC/SD ratio), the Kolmogorov-Smirnov test was used. This informed the choice of appropriate statistical tests for the subsequent analyses. The Mann-Whitney test, a non-parametric method, was used to compare the values of PC, SD, and their ratio between participants with and without EVs. This test was chosen due to the non-normal distribution of the variables. The diagnostic efficacy of PC, SD, and PC/SD ratio in predicting the presence of EVs was evaluated with the use of receiver operating characteristic (ROC) curve analysis. Youden's J index was used to identify the ideal cut-off values, and the resulting sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were computed. The data analyses were conducted using SPSS version 16, MedCalc Version 19.5.3, and GraphPad Prism version 8.0.1 software. The significance level was set at 0.05.

Results

The study included a total of 147 patients diagnosed with cirrhosis. The mean age of participants was 56.18 ± 11.98 years, with 16 patients (10.9%) being older than 70 years. The majority of the study population was male (61.9%) and married (77.6%). The average duration of the disease among the participants was 3.31 ± 1.93 years, and 43 patients (29.3%) had a disease duration of more than four years (Table 1).

The most frequent underlying causes for cirrhosis were non-alcoholic steatohepatitis (NASH) (23.8%), hepatitis C virus (HCV) (19.7%), and alcohol-related (17.0%) liver disease. Based on the Child-Pugh classification, 31 patients (21.1%) were categorized as Class A, 81 (55.1%) as Class B, and 35 (23.8%) as Class C. EVs were discovered in 108 (73.5%) of the 147 patients during endoscopic screening. Interestingly, the study found that the average duration of cirrhosis was significantly longer in patients with EVs compared to those without (p<0.001). While the group with EVs had a larger percentage of women (65.7%) than the group without EVs (51.3%), the difference in percentages between the two groups was not statistically significant (p=0.111) (Table 2).

Table 1. Demographic and clinical characteristics of patients with cirrhosis presenting to the Razi Hospital of Rasht in 2023.

Variable	Total (n=147)	with EVs (n=108)	without EVs (n=39)	P value
Age (year)				
≤ 50	48 (32.7)			
50-70	83 (56.5)			
> 70	16 (10.9)			
Mean (SD)	56.18 (11.98)	56.62 (11.65)	54.95 (12.93)	0.457
Sex				
Male	91 (61.9)	37 (34.3)	19 (48.7)	0.111
Female	56 (38.1)	71 (65.7)	20 (51.3)	
Marital Status				
Single	33 (22.4)	19 (17.6)	14 (35.9)	0.019
Married	114 (77.6)	89 (82.4)	25 (64.1)	
Duration of disease (year)				
≤ 2	59 (40.1)			
3-4	45 (30.6)			
> 4	43 (29.3)			
Mean (SD)	3.31 (1.93)	3.64 (1.91)	2.38 (1.68)	<0.001

Table 2. Comparison of platelet count (PC), spleen diameter (SD), and platelet count to spleen diameter (PC/SD) ratio between individuals with and without esophageal varices in patients with cirrhosis.

Variable	Total (N=147)	Individuals without EVs (N=39)	Individuals with EVs (N=108)	P value
PC (n/mm^3)	85000 (69000-105000)	105000 (83000 - 113000)	81000 (65250 - 96000)	<0.001
SD (mm)	160 (150-175)	155 (130 - 160)	162 (155 - 180)	<0.001
PC/SD ratio	533.7 (418.2-687.5)	652 (550 - 942)	484 (389 - 625)	<0.001

To evaluate these parameters' ability to predict the diagnosis of EVs, ROC analysis was performed. The area under the ROC curve (AUROC) for PC was 0.695 (95% CI: 0.603-0.787), for SD 0.750 (95% CI: 0.663-0.837), and the PC/SD ratio 0.734 (95% CI: 0.646-0.822) (Figure 1).

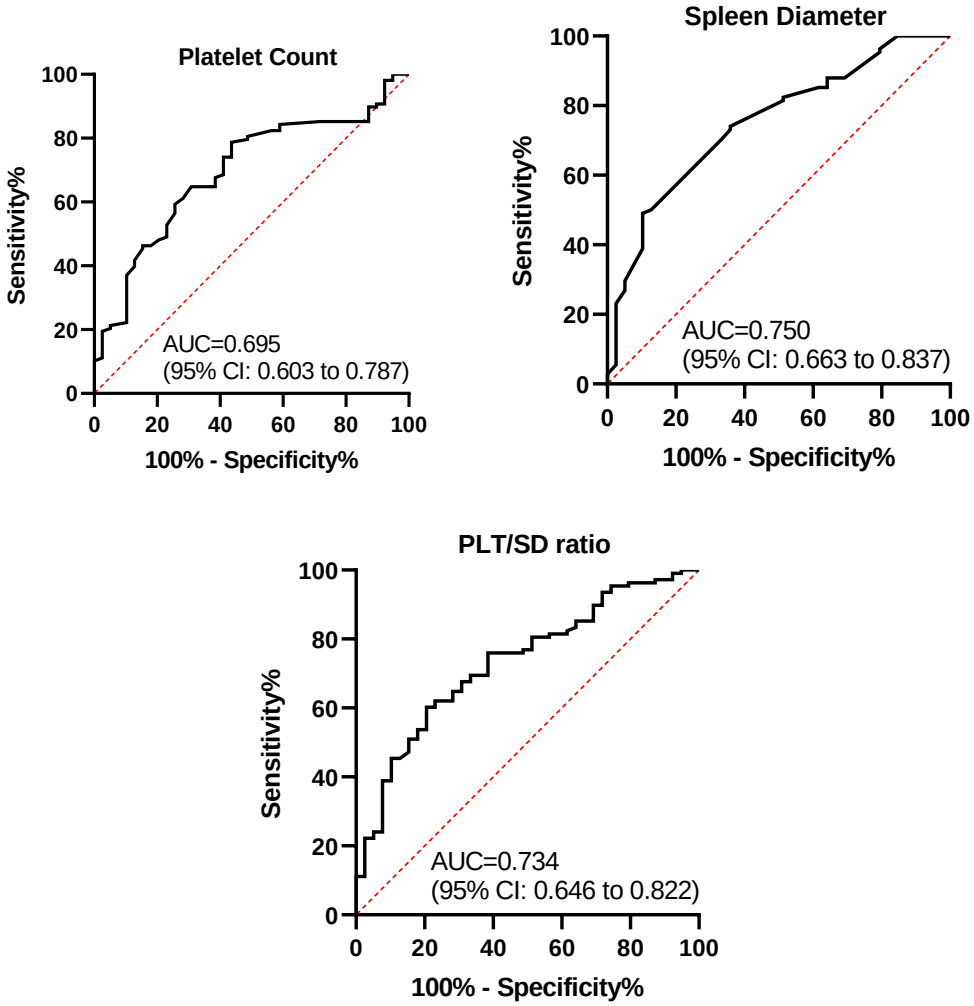

Figure 1. The predictive power of platelet count (PLT), spleen diameter (SD), and the ratio of platelet count to spleen diameter (PLT/SD Ratio) in the diagnosis of esophageal varices using the area under the curve (ROC).

AUC: Area Under Curve. CI: Confidence Interval

The following is the determination of the ideal cut-off values: PC > 100,000 (sensitivity 78.7%, specificity 56.4%), SD < 163 mm (sensitivity 49.1%, specificity 89.7%), and PC/SD ratio ≥ 523 (sensitivity 60.2%, specificity 79.5%).

Patients with a PC/SD ratio below the cut-off value of 523, a PC below the cut-off of 100,000, and an SD above the cut-off of 163 mm were more likely to have EVs. The PPV of these cut-off values were 89%, 83.3%, and 93%, respectively (Table 3).

Table 3. The best cut-off points of platelet count, spleen diameter, and platelet count to spleen diameter ratio in the diagnosis of esophageal varices.

	PC (n/mm^3)	SD (mm)	PC/SD ratio
Area under curve	0.695	0.750	0.734
Best cutoff point	≤ 100000	> 163	≤ 523
Sensitivity	78.7	49.1	60.2
specificity	56.4	89.7	79.5
Positive predictive value	83.3	93.0	89.0
Negative predictive value	48.9	38.9	41.9
Positive likelihood value	1.81	4.78	2.93
Negative likelihood value	0.38	0.57	0.50

Discussion

The present investigation aimed to evaluate the prevalence of EVs among cirrhotic patients and assess the utility of PC, SD, and the PC/SD ratio in predicting the presence of EVs. The study found that 108 (73.5%) of the 147 participants had EVs. According to the study results, cirrhotic individuals with EVs had considerably smaller PC, greater SD, and lower PC/SD ratios than those without EVs. Furthermore, the diagnostic utility of these parameters in predicting EVs was assessed using ROC analysis.

The prevalence of EVs in this study was consistent with those from other areas, such as southern India (77.7%), Mexico (80.2%), and China (74.7%) (10-12). However, lower prevalence rates were reported in studies conducted in Tanzania (39.5%) and South Carolina (51%) (13, 14). This variation in the occurrence of EVs across different patient populations could be attributed, in part, to differences in the underlying causes of liver cirrhosis. For instance, patients with biliary cirrhosis exhibited a lower prevalence of EVs (26.0%), while those with hepatitis B-related liver cirrhosis had a considerably higher rate (74.7%) (12, 15).

The results of the present investigation suggest that SD may represent a more reliable individual non-invasive marker for the prediction of EVs compared to PC or the PC/SD ratio in the study population, as evidenced by the AUROC values reported herein. These findings contrast with the conclusions of certain prior studies, which have proposed the PC/SD ratio as a more accurate non-invasive marker relative to PC or SD individually (8, 9).

The predictive power of the PC/SD ratio in the current study was satisfactory but not optimal. However, a number of previous investigations have documented higher discriminative ability of this marker (5, 16-18). Specifically, Giannini et al., who first introduced the PC/SD ratio as a promising non-invasive tool, reported an AUROC of 0.86 in predicting the presence of EVs (17). Similarly, Patil et al. observed an AUROC of 0.84 for the PC/SD ratio, a value exceeding that obtained in the present investigation (18).

The differences in the diagnostic utility of these non-invasive markers for predicting EVs across studies can be attributed to several factors. Firstly, the study populations may have varied in terms of the underlying etiologies of cirrhosis, disease severity, and the prevalence of EVs. Secondly, the cut-off values used for PC, SD, and PC/SD ratio varied across studies, which can affect the sensitivity and specificity of these

parameters in predicting the presence of EVs. Furthermore, the discrepancies observed in the diagnostic performance of these non-invasive parameters may be partially attributed to the influence small sample size of this study.

Despite the mixed findings, the present study demonstrated that the PC/SD ratio had a high PPV of 93%, indicating that patients with a ratio below the cut-off are highly likely to have EVs. However, the relatively low NPV of 41.9% suggests that a ratio above the cut-off may not accurately exclude the presence of EVs.

This emphasizes the potential utility of the PC/SD ratio as a screening tool for identifying high-risk patients. By employing this method, healthcare providers can effectively stratify patients according to their risk levels, facilitating a more focused approach to endoscopic screening and monitoring. This prioritization is crucial, as it enables clinicians to concentrate their resources and efforts on individuals who are most likely to benefit from early intervention.

The ability of a non-invasive predictor to accurately identify high-risk patients can help prevent serious complications, such as variceal hemorrhage, which is vital in managing conditions like cirrhosis. Early intervention not only enhances patient outcomes by averting adverse events but also improves the overall quality of care provided.

Moreover, this targeted approach contributes to the efficient allocation of healthcare resources. By ensuring that high-risk individuals receive timely care, healthcare systems can minimize unnecessary procedures for patients at lower risk, thereby alleviating the burden on medical facilities and personnel. This efficiency is particularly important in environments where healthcare resources are constrained, as it allows for better management of patient loads and enhances the overall effectiveness of the healthcare system.

It is imperative to acknowledge that although the PC/SD ratio exhibits potential as a non-invasive marker for predicting EVs and can assist in prioritizing patients for endoscopy, it is crucial to emphasize that it cannot replace traditional endoscopic procedures. Although previous studies have reported a high predictive ability for this marker (5, 16-18), our findings did not achieve that level of performance, indicating that its effectiveness was not optimal in this context. Therefore, the use of the PC/SD ratio should be approached with caution until sufficient evidence supports their efficacy.

The most reliable method for identifying EVs and determining their severity is still endoscopy, as it allows for direct visualization and grading of the varices (19).

In addition to the PC/SD ratio, other non-invasive indicators have been explored for the prediction of EVs in cirrhotic patients, such as various serum biomarkers (20-22). The combination of these biomarkers with the PC/SD ratio may further improve the diagnostic accuracy in predicting the presence of EVs, and this should be investigated in future studies.

The current research has certain limitations. Firstly, the fact that the study was limited to a single tertiary care facility may limit the applicability of the findings in other contexts. Secondly, the cross-sectional design of the study precluded the assessment of the long-term predictive value of the PC/SD ratio in identifying the development or progression of EVs. Prospective longitudinal studies would be valuable in evaluating the utility of the PC/SD ratio for monitoring the risk of EVs over time.

Conclusion

In conclusion, the present study suggests that PC, SD, and PC/SD ratio can be considered as beneficial non-invasive markers for predicting the presence of EVs in patients with hepatic cirrhosis. These parameters may help identify individuals who should prioritize undergoing upper gastrointestinal endoscopy for EV screening. However, comprehensive endoscopic examination should remain the standard approach for the identification and treatment of EVs in cirrhotic patients.

Author contribution

Concept development (provided idea for the research): **SKHGh** and **SF** Design (planned the methods to generate the results): **SM**, **SKHGh**, **FJ**, **ASh** Supervision (provided oversight, responsible for

organization and implementation): **FJ**, **AH** and **NL** Data collection/processing (responsible for experiments, patient management, organization, or reporting data) and data analysis/interpretation (responsible for statistical analysis, evaluation, and presentation of the results): **NL**, **AH**, **SKHGh**, **SF** Literature search (performed the literature search and writing of the manuscript): **NL**, **AH** and **SKHGh** Drafting the manuscript (responsible for writing a substantive part of the manuscript): All authors.

Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

This study was approved by the ethics committees of the Guilan University of Medical Sciences [IR.GUMS.REC.1403.052]. Informed consent was obtained from all individual participants

References

1. Al-Busafi SA, et al. Clinical manifestations of portal hypertension. Int J Hepatol. 2012;2012:203794.

2. Garcia-Tsao G, et al. Portal hypertensive bleeding in cirrhosis: Risk stratification, diagnosis, and management: 2016 practice guidance by the American Association for the study of liver diseases. Hepatology. 2017;65(1):310-35.

3. Gralnek IM, et al. Challenges in the Management of Esophagogastric Varices and Variceal Hemorrhage in Cirrhosis – A Narrative Review. The American Journal of Medicine. 2024;137(3):210-7.

4. Verma A, et al. Sudden Death Caused by Gastroesophageal Varices Rupture: Insights From an Autopsy-Based Case Series Unraveling the Pathological Events. Cureus. 2023;15(9):e46166.

5. Sarangapani A, et al. Noninvasive prediction of large esophageal varices in chronic liver disease patients. Saudi J Gastroenterol. 2010;16(1):38-42.

6. Garcia-Tsao G, et al. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. Hepatology. 2007;46(3):922-38.

7. Pallio S, et al. Diagnosis and Management of Esophagogastric Varices. Diagnostics (Basel). 2023;13(6).

8. Barrera F, et al. Platelet count/spleen diameter ratio for non-invasive prediction of high risk esophageal varices in cirrhotic patients. Ann Hepatol. 2009;8(4):325-30.

9. ElDesoky AE, et al. Platelet count/spleen diameter ratio as a predictor of high-risk esophageal varices in patients with liver cirrhosis. Medical Journal of Viral Hepatitis. 2022;6.2(2):25-30.

10. González-Ojeda A, et al. Platelet count/spleen diameter ratio to predict esophageal varices in Mexican patients with hepatic cirrhosis. World J Gastroenterol. 2014;20(8):2079-84.

11. Cherian JV, et al. Non-invasive predictors of esophageal varices. Saudi J Gastroenterol. 2011;17(1):64-8.

12. Hong WD, et al. Predictors of esophageal varices in patients with HBV-related cirrhosis: a retrospective study. BMC Gastroenterol. 2009;9:11.

13. Madhotra R, et al. Prediction of esophageal varices in patients with cirrhosis. J Clin Gastroenterol. 2002;34(1):81-5.

14. Gunda DW, et al. The magnitude and correlates of esophageal Varices among newly diagnosed cirrhotic patients undergoing screening fibre optic endoscope before incident bleeding in North-Western Tanzania; a cross-sectional study. BMC Gastroenterology. 2019;19(1):203.

15. Levy C, et al. Prevalence and predictors of esophageal varices in patients with primary biliary cirrhosis. Clin Gastroenterol Hepatol. 2007;5(7):803-8.

16. Baig WW, et al. Platelet count to spleen diameter ratio for the diagnosis of esophageal varices: Is it feasible? Can J Gastroenterol. 2008;22(10):825-8.

17. Giannini EG, et al. Platelet count/spleen diameter ratio for the noninvasive diagnosis of esophageal varices: results of a multicenter, prospective, validation study. Am J Gastroenterol. 2006;101(11):2511-9.

18. Patil S, et al. Platelet Count/Spleen Diameter Ratio as a Non-Invasive Predictor of Esophageal Varices in Cirrhotic Patients: A Single-Center Experience. Gastroenterology Insights [Internet]. 2024; 15(1):[98-106 pp.].

19. Shrestha R, et al. Endoscopic Detection and Management of Esophagogastric Varices. Cureus. 2021;13(8):e16825.

20. Duah A, et al. Non-invasive markers as predictors of oesophageal varices in cirrhotic patient in a teaching hospital in Ghana. Ghana Med J. 2019;53(2):142-9.

21. Li S, et al. Assessment of Non-invasive Markers for the Prediction of Esophageal Variceal Hemorrhage. Front Med (Lausanne). 2021;8:770836.

22. Glisic T, et al. Diagnostic Value of Non-invasive Scoring Systems in the Prediction of Esophageal Varices in Patients with Liver Cirrhosis-Single Center Experience. Medicina (Kaunas). 2022;58 (2).

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Case report Free Access

Malignant transformation of multiple exostosis: a case report

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Abstract

Introduction: Osteochondroma is a benign tumor of bone. Malignant transformation of Osteochondroma is the most devastating complication one can encounter. Osteochondroma can transform into any malignancy like Osteosarcoma, Chondrosarcoma and Ewing sarcoma. Malignant transformation is more common in patients with multiple exostosis. Recognition of this malignant transformation is needed to predict the patient's outcome.

Case presentation: A 26-year-old male patient came with complaints of a mass in the left knee region for the past 7 years. X-ray of the knee showed multiple pedunculated exostosis on either side of the distal end of the femur, tibia and fibula. Histopathological examination revealed a bony lesion with a cartilaginous cap of increased thickness and cellularity. The cartilaginous cap possesses plump chondrocytes showing binucleation-forming nodules with mild atypia. The cartilaginous cap undergoes endochondral ossification, suggesting the possibility of a secondary peripheral atypical cartilaginous tumor from osteochondroma of the tibia.

Discussion: Chondrosarcoma is a heterogeneous type of primary bone cartilaginous malignancy with variable clinical outcomes. Malignant transformation of osteochondroma in the appendicular skeleton was named atypical cartilaginous tumor; in the axial skeleton, it is named Grade 1 Chondrosarcoma.

Conclusion: Differentiation between osteochondroma and its malignant transformation can be possible if made in a multidisciplinary setting such as clinical history, radiological findings along with histology to confirm the diagnosis.

Keywords: Osteochondroma, Chondrosarcoma, Bone tumour

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Introduction

Osteochondroma the most common benign tumour of bone accounts for about 35% of benign bone tumours affecting 3% of the population (1-3). Osteochondroma arises from the metaphysis of bones most commonly in the second to third decade of life. Commonly affected bones are long bones of the leg, scapula and pelvis (4).

Osteochondroma usually presents as a painless, asymptomatic mass and is usually found as an incidental finding. Osteochondroma are benign cartilage forming tumor derived from aberrant cartilage of the perichondral ring that may present either as solitary osteochondroma or multiple hereditary exostosis leading to syndromic manifestation of the lesion (5,6).

Osteochondromas are mostly treated by surgical excision of the lesion either partially or completely. The most common complication of osteochondroma is its malignant transformation. Osteochondroma can transform into osteosarcoma, chondrosarcoma and Ewing sarcoma (7,8). Malignant transformation of osteochondroma into chondrosarcoma is considered as drastic complication of osteochondroma accounting for about less than 1% of the cases (9). 3-5% of patients with multiple osteochondromas undergo malignant transformation (10). Here we present a case of a young male with multiple exostosis presenting with malignant transformation.

Case presentation

A 26-year-old male patient came with complaints of pain in the left knee for the past 7 years. The patient took medications for 3-4 years as analgesics, but after 4yrs since patient was suffering from more pain and swelling over the knee joint, he took X-ray, X-ray showed mass in the left knee region. History of pain during rest and walking. The swelling was insidious in onset, progressive in nature, not mobile, hard in consistency, fixed to the underlying bone. No history of any previous surgery or chemo or radiotherapy.

The patient had undergone radiological examination and X-ray knee showed multiple pedunculated exostosis noted on either side of distal end of femur, Proximal end of tibia and fibula (Figure 1).

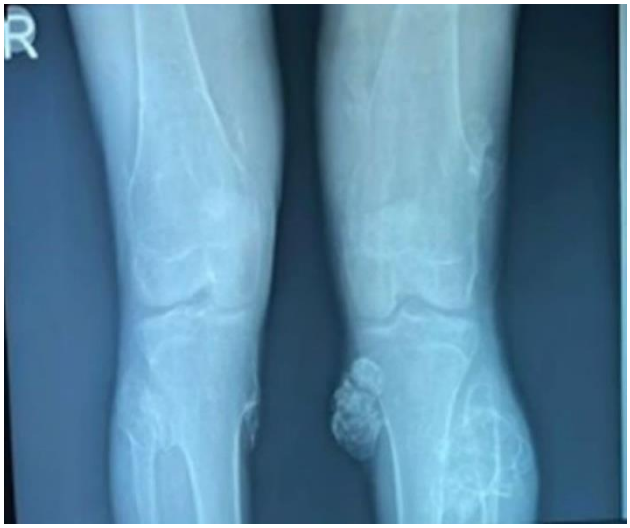

Figure 1. Xray image of the patient showing lobulated mass over tibia and fibula.

Reconstructed 3D imaging showed multiple sessile and pedunculated exostosis noted in multiple visualized bones, largest measuring 6.2 x 6.2 x 6.6cm. Pedunculated metaphyseal exostosis away from joint space in the medial aspect of proximal tibia with calcification of chondroid matrix, suggesting the possibility of Osteochondroma with sarcomatous transformation. (Figure 2). There is no significant family history of any bone lesions.

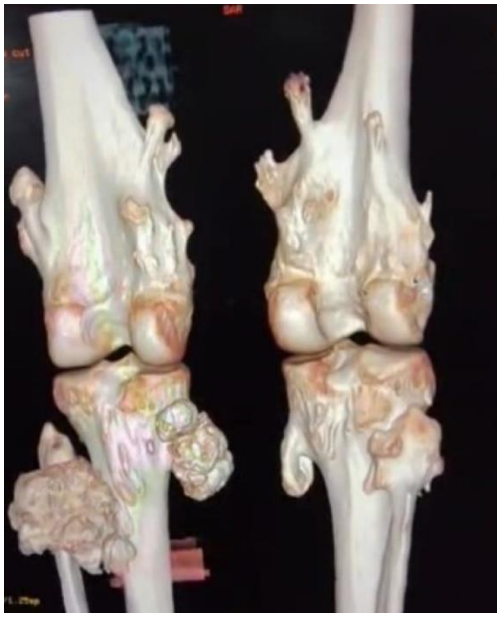

Figure 2. Reconstructed 3D image of the patient showing multiple pedunculated mass over the tibia and fibula.

Excision of a single pedunculated mass from the lateral aspect of tibia was received which showed a single bony tissue with a cartilaginous cap totally measuring

6 x 3.5 x 4 cm. Cut surface shows bone tissue measuring 3.5 x 2.5cm with irregular nodular cartilage cap of varying thickness measuring 2.5cm at its thickest portion permeating into the bony stalk (Figure 3).

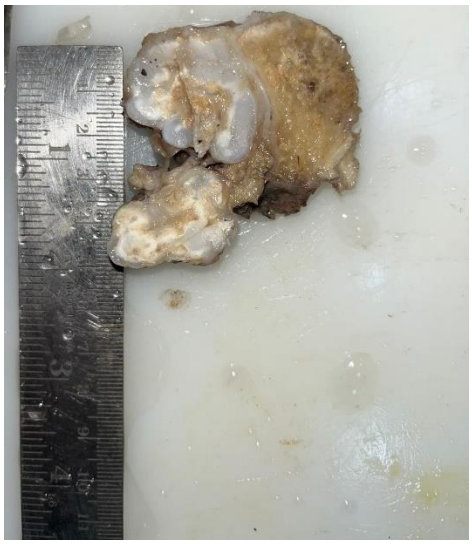

Figure 3. Bony stalk with cartilaginous cap of varying thickness and permeation into the bone.

Histopathological examination revealed a bony lesion with cartilaginous cap of increased thickness and cellularity. Cartilaginous cap increased cellularity possess plump chondrocytes showing binucleation forming nodules with mild nuclear enlargement, irregularity and atypia. Cartilaginous cap undergoes endochondral ossification as in a case of osteochondroma, suggesting the possibility of Secondary peripheral atypical cartilaginous tumor from osteochondroma of tibia (Figure 4,5).

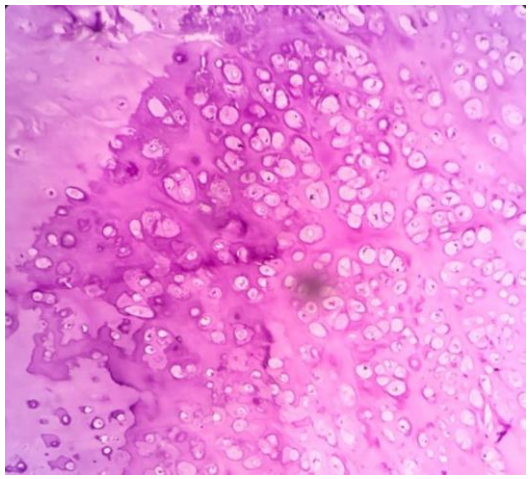

Figure 4. Histopathological image showing cartilage undergoing endochondral osssification (H&E stain, 10X).

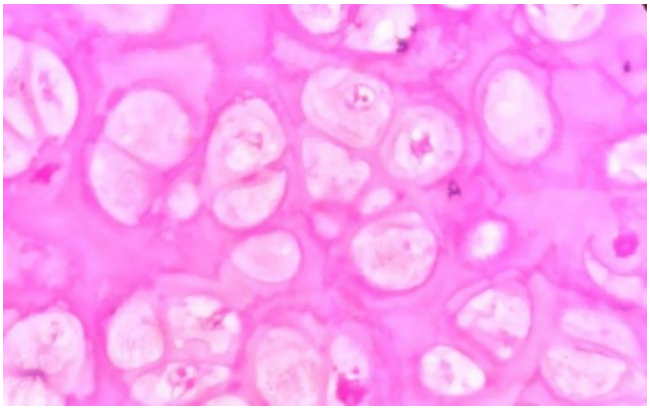

Figure 5. Histopathological image showing nodules of chondrocytes exhibiting mild atypia (H&E stain, 40X).

Discussion

Hereditary multiple osteochondromas (HMO), an autosomal dominant disorder involves two or more exostoses in the axial or appendicular skeleton. It is diagnosed by presence of two or more osteochondromas, detected radiographically in the metaphyseal ends of the long bones (11).

Chondrosarcoma is a heterogeneous type of primary bone cartilaginous malignancy with variable clinical outcomes (12). These are locally aggressive, hyaline cartilage-producing neoplasm arising within the cartilaginous cap of a pre-existing osteochondroma, tumours in the appendicular skeleton can be called as peripheral atypical cartilaginous tumor and tumours of the axial skeleton (including the pelvis, scapula, and skull base) can be called peripheral chondrosarcoma Grade 1 (13).

The incidence of chondrosarcoma varies between various bones with Ileum (19%), followed by the scapula (15%), pubic bone (10%), ribs (10%), tibia (12%) and femur (11%) (13).

Patients with multiple osteochondromas carrying germline mutations in EXT1 or EXT2 are at increased risk of developing ACT/CS1 within the cartilaginous cap of osteochondromas. Malignancy risk in case of multiple osteochondromas is as high as about 5% when compared to solitary osteochondromas which is about 1% (14).

Functional loss of genes EXT1 and EXT2 encoding glucosyltransferases which is involved in the synthesis of heparan sulfate causes Hereditary multiple exostosis

(HME). HME genetic transmission occurs in autosomal dominant pattern or loss of heterozygosity or haploinsuffiency or through mutations in post-transcriptional regualtory pathways.

Even isolated mutations of EXT1 and EXT2 gene cause pathology affecting the patient's growth. Malignant transformation is usually rare accounting for about 2 to 4% in patients affected by HME. A well-differentiated carcinoma is usually diagnosed, but very rarely osteosarcomas and dedifferentiated chondrosarcomas from bone could arise (15).

Differentiation between osteochondroma and its malignant transformation can be possible if made in a multidisciplinary setting such as clinical history, radiological findings along histology to confirm the diagnosis (14).

Treatment of Multiple exostosis is surgical removal, especially in symptomatic cases irritating adjacent structures. Though the treatment strategies are limited, precise diagnosis is essential for management. In Future, molecular analysis of EXT1 and EXT2 genes is essential for understanding the disease at molecular and cellular level and reveals new treatment options or therapeutic targets in both Hereditary multiple exostosis and chondrosarcoma (15).

Chemotherapy and radiation are not indicated for chondrosarcoma since they are resistant to both. Grade I chondrosarcoma with minimal rate of metastasis in the extremities are treated by intralesional curettage, high speed burring and adjuvant treatment with internal fixation and packing using phenol or ethanol or liquid nitrogen. Lesions in pelvis or axial skeleton needs wide local excision (15).

The 5-year and 10-year local recurrence rates for secondary peripheral chondrosarcoma are 15.9% and 17.5% respectively. The 5-year and 10-year mortality rates are 1.6% and 4.8% respectively. Local recurrences are possible due to incomplete excision in inaccessible locations (14).

Conclusion

This case report deals with the most common bone tumour osteochondroma undergoing malignnat transformation which emphasize the fact that multiple disciplinary evaluation, as well as careful gross examination, helps us to make the proper diagnosis at the appropriate time which helps in improving the prognosis and outcome of the patient.

We hope that this case report raises awareness among clinicians and pathologists to this possible transformation of osteochondroma to chondrosarcoma, and that thorough investigation drives further development in the diagnosis and safe treatment for improving patient outcomes.

Author contribution

BB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing original draft. **SK**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing original draft. **JS**: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing original draft, Writing review & editing

Conflict of interest

The authors declare that they have no competing interests.

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References

1. Kitsoulis P, Galani V, Stefanaki K, et al. Osteochondromas: review of the clinical, radiological and pathological features. In Vivo. 2008;22(5):633-46.

2. Morton KS. On the question of recurrence of osteochondroma. J Bone Joint Surg Br. 1964;46(4):723-5.

3. Saglik Y, Altay M, Unal VS, Basarir K, Yildiz Y. Manifestations and management of osteochondromas: a retrospective analysis of 382 patients. Acta Orthop Belg. 2006;72(6):748- 55.

4. Tianjun Lan, Xin Liu, Pei-Sheng Liang,Qian Tao. Osteochondroma of coronoid process: A case report send review of literature. Oncol Lett.2019;18:2270-2277.

5. Vikram V Kadu, Saindane KA, Ninad Goghate, Neha Goghate. Osteochondroma of the Rib: A rare radiological appearance. J Orthop Case Rep. 2015;5:62- 64.

6. Dr. Gyneshwar Tank, Dr. Sumit Agarwal, Dr. Kalom Jamoh. Osteochondroma transform into secondary low-grade chondrosarcoma with similar histology features: Case report. Int J Case Rep Orthop 2022;4(2):09.

7. Moradi Tabriz H, Obohat M, Vahedifard F, Eftekharjavadi A. Survey of Mast Cell Density in Transitional Cell Carcinoma. Iran J Pathol. 2021;16(2):119-27.

8. Salari S, Ghadyani M, Karimi M, Mortezazadeh M, Vahedifard F. Immunohistochemical Expression Pattern of MLH1, MSH2, MSH6, and PMS2 in Tumor Specimen of Iranian Gastric Carcinoma Patients. Journal of Gastrointestinal Cancer. 2022;53(1):192-6:10.

9. Kuruwitaarachchi, Kasun & Munidasa, Dilshan.Secondary chondrosarcoma from a solitary osteochondroma of the fibula head: a case report. Journal of the Postgraduate Institute of Medicine.2021.8:135.

10. Tsuda, Yusuke & Gregory, Jonathan & Fujiwara, Tomohiro & Abudu, Seggy. Secondary chondrosarcoma arising from osteochondroma: outcomes and prognostic factors. The bone & joint journal. 2019:101-B. 1313-1320.

11. Vlok SCS, Wagener GWW, Zaharie D. Secondary chondrosarcoma: Malignant transformation of pre-existing hereditary and nonhereditary cartilaginous lesions. S Afr J Rad. 2014;18.

12. Arsenault M, Alam W, Lambert T, Li S, Adorno DN, et al. Secondary Chondrosarcoma Arising from Osteochondroma: Case Report and Literature Review. JSM Clin Cytol Pathol 2019: 4:3.

13. Sbaraglia M, Bellan E, Dei Tos AP. The 2020 WHO Classification of Soft Tissue Tumours: news and perspectives. Pathologica. 2021 Apr;113(2):70-84.

14. Goldblum JR, Lamps LW, Mckenney JK, Myers JL, Rosai J. Rosai and Ackerman's Surgical Pathology. Philadelphia, Pa: Elsevier; 2018.

15. Ottesen TD, Shultz BN, Munger AM, Amick M, Toombs CS, Friedaender GE, Grauer JN. Chondrosarcoma patient characteristics, management, and outcomes based on over 5,000 cases from the National Cancer Database (NCDB). PLoS One. 2022 Jul 28;17(7):e0268215.

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Alpha-fetoprotein as a predictor of liver disease progression in HBV patients with HIV and HCV co-infections

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Abstract

Introduction: Hepatitis B virus (HBV) infection is a significant health challenge globally, especially in sub-Saharan Africa. Co-infections with HIV and HCV worsen HBV-related liver diseases, complicating clinical management. Alpha-fetoprotein (AFP) is a key biomarker for monitoring liver disease progression and detecting hepatocellular carcinoma (HCC). This study evaluates AFP levels in HBsAg and HBeAg seropositive patients with and without HIV and HCV co-infections over one year in Warri, Delta State, Nigeria. This study aimed to understand the impact of HIV and HCV co-infections on liver disease prognosis in HBV patients by evaluating AFP levels and liver function over one year.

Materials and Methods: This longitudinal cohort study included 200 HBsAg and HBeAg seropositive patients aged 18-65 years, divided into three groups: HBV monoinfection (n=80), HBV/HIV co-infection (n=60), and HBV/HCV co-infection (n=60). Participants were followed for one year with quarterly blood sample collections for AFP measurement using ELISA, liver function tests (ALT, AST, ALP, bilirubin), and viral load assessments. Sociodemographic data were also collected.

Results: AFP levels were significantly higher in the HBV/HCV co-infection group (36.2 ± 12.4 ng/mL) compared to the HBV monoinfection (12.5 ± 4.3 ng/mL) and HBV/HIV co-infection groups (18.7 ± 6.8 ng/mL) ($p<0.001$). Elevated liver function tests, particularly ALT and AST, were more prevalent in the HBV/HCV co-infection group. AFP levels positively correlated with ALT ($r=0.52$, $p<0.01$) and AST ($r=0.47$, $p<0.01$) in the HBV/HCV co-infection group.

Conclusion: The higher AFP levels in HBV/HCV co-infected patients indicate an increased risk of liver disease progression and HCC. The positive correlations between AFP and liver enzymes suggest ongoing liver damage and regeneration in this group. These findings underscore the importance of routine AFP and liver function tests in the early detection and treatment of liver disease among HBV patients, particularly those with HCV co-infection, to enhance clinical outcomes.

Keywords: Alpha-fetoprotein (AFP), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Liver function tests, Hepatocellular carcinoma, Co-infection

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Introduction

Hepatitis B virus (HBV) infection continues to be a significant global health challenge, particularly in sub-Saharan Africa, where the prevalence remains high (1, 2). Complicating the clinical landscape, co-infections with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) are common, exacerbating the progression of liver diseases associated with HBV. Co-infections pose additional challenges in clinical management and prognosis, necessitating more in-depth studies to understand their interactions and effects on liver health (1, 2).

Alpha-fetoprotein (AFP) serves as a crucial biomarker in monitoring liver disease progression and detecting hepatocellular carcinoma (HCC). Elevated AFP levels are often associated with liver inflammation, regeneration, and malignancy (3). Despite its widespread use, the dynamics of AFP levels in HBV patients with concurrent HIV and HCV infections remain inadequately explored(4). This study focuses on evaluating AFP levels in HBsAg and HBeAg seropositive patients, both with and without HIV and HCV co-infections, over one year in Warri, Delta State, Nigeria. The findings aim to provide insights into the impact of these viral interactions on liver disease prognosis and AFP variability (3–6).

In-depth knowledge of the implications of anti-HBe in HBV infection is crucial for comprehensive disease management. The presence of hepatitis B e-antigen (HBeAg) in the blood typically indicates active viral replication and high infectivity (7). Conversely, the appearance of antibodies against HBeAg (anti-HBe) usually suggests a transition to a lower replicative state of the virus, which is often associated with a more favorable prognosis (8–11). However, this seroconversion does not necessarily mean that the virus has been cleared from the liver. It signifies that the immune system has responded to the virus in a way that reduces its replication (7–11).

Liver disease in HBV patients, particularly in those with co-infections, poses a complex challenge for clinical management (12–14). Co-infection with HIV and HCV can alter the natural course of HBV infection, leading to more severe liver damage and an increased risk of HCC. HIV co-infection, for instance, can accelerate the progression of liver fibrosis and increase the likelihood of cirrhosis and liver-related mortality. Similarly, HCV co-infection can result in more aggressive liver disease and complicate treatment outcomes. Therefore, scientific knowledge on how these co-infections influence AFP levels and liver disease progression is vital for improving patient outcomes (12–14).

The primary objective of this study is to assess the levels of AFP in HBsAg and HBeAg seropositive patients, with and without HIV and HCV co-infections, over one year. This evaluation will help elucidate the influence of co-infections on liver disease progression and the potential development of HCC. By examining AFP levels longitudinally, this research aims to highlight any significant fluctuations that could be indicative of disease progression or response to therapy (15-17).

Materials and methods

Study Design and Population

This longitudinal cohort study was conducted over one year, involving 200 HBsAg and HBeAg seropositive patients aged 18-65 years, recruited from healthcare facilities in Warri, Delta State, Nigeria.

Sample Size Determination

The sample size was calculated using the formula:

$n = Z^2 * P(1-P) / d^2$

Where:

n = required sample size

Z = 1.96 (for 95% confidence level)

P = 0.109 (10.9% prevalence) (18)

d = 0.05 (5% precision)

$n = (1.96)^2 * 0.109(1-0.109) / (0.05)^2$

n = 3.8416 * 0.109 * 0.891 / 0.0025

n = 149.82

Rounding up to the nearest whole number: 150

To account for potential non-response or dropout, 15% proportion was added to the minimum sample size obtained

150 + (150 * 0.15) = 172.5

Accordingly, a minimum sample size of 173 subjects was appropriate for the study to achieve a 95% confidence level with 5% precision. However, to improve diversity in study participation and to ensure greater precision, 200 subjects were recruited for the study (19).

The study population was categorized into three groups:

1. HBV monoinfection without HBeAb expression (n=80)
2. HBV/HIV co-infection without HBeAb expression (n=60)
3. HBV/HCV co-infection without HBeAb expression (n=60)

Inclusion and Exclusion Criteria

Participants were included if they were seropositive for HBsAg and HBeAg and had no expression of HBeAb. Exclusion criteria included prior liver disease, HCC, or other significant co-morbidities.

Ethical Considerations

This study obtained Ethical Approval from the Delta State Ministry of Health Research and Ethics Review Committee. We adhered to ethical principles, including:

i. Informed Consent: Each participant received a written informed consent form alongside the questionnaire, ensuring their consent to participate.

ii. Data Confidentiality: Findings from the study were kept confidential and shared only among co-investigators.

iii. Beneficence: The results of the findings were provided to the managing clinical team without any charge.

iv. Voluntariness: Both cases and controls had the option to decline participation in the study when approached.

Data Collection

Blood samples were collected quarterly for one year. Alpha-fetoprotein (AFP) levels were measured using enzyme-linked immunosorbent assay (ELISA). Additional tests included liver function tests (ALT, AST, ALP, and bilirubin) and viral load assessments. Sociodemographic data, including age, gender, occupation, and lifestyle factors, were collected using structured questionnaires.

Principles of Assays for Laboratory Analysis

Alpha-fetoprotein (AFP) Measurement

AFP levels were determined using a commercial Enzyme-Linked Immunosorbent Assay (ELISA) kit (Bio-Rad kit). The principle of ELISA involves the following steps:

1. **Antigen-Antibody Binding:** The AFP in the patient's sample binds to the specific antibodies coated on the wells of the ELISA plate.
2. **Washing:** Unbound substances are removed through washing.
3. **Enzyme-Linked Secondary Antibody:** An enzyme-linked secondary antibody specific to AFP is added, which binds to the AFP already captured by the primary antibody.
4. **Substrate Addition:** A substrate is added that the enzyme converts to a detectable signal, typically a color change.
5. **Detection:** The intensity of the color is measured using a spectrophotometer and is proportional to the AFP concentration in the sample.

Liver Function Tests

Liver function tests (LFTs) including ALT, AST, ALP, and bilirubin levels were measured using automated

biochemical analyzers (Biobase BS-230). The principles of these tests are as follows:

1. **Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST):**
    - **Enzyme Activity Measurement:** ALT and AST catalyze the transfer of amino groups from alanine and aspartate to alpha-ketoglutarate, respectively. The reaction produces pyruvate and oxaloacetate, which are then converted to a detectable product.
    - **Spectrophotometry:** The change in absorbance is measured, reflecting enzyme activity.
2. **Alkaline Phosphatase (ALP):**
    - **Enzyme Activity Measurement:** ALP catalyzes the hydrolysis of phosphate esters, releasing inorganic phosphate.
    - **Spectrophotometry:** The release of phosphate is measured, indicating enzyme activity.
3. **Bilirubin:**
    - **Direct and Total Bilirubin Measurement:** Bilirubin reacts with diazo reagent to form azobilirubin, which is measured spectrophotometrically.
    - **Indirect Bilirubin Calculation:** Indirect bilirubin is calculated by subtracting direct bilirubin from total bilirubin.

HBsAg, HBeAg, Anti-HBe, Anti-HCV, and HIVp24 detection

These markers were measured using ELISA kits (Bio-Rad kit), and the principles are similar to the AFP ELISA described above:

1. **Antigen/Antibody Binding:** The specific antigen or antibody in the patient's sample binds to the corresponding antibody or antigen coated on the ELISA plate.
2. **Washing:** Unbound components are washed away.
3. **Enzyme-Linked Secondary Antibody:** An enzyme-linked secondary antibody specific to the target antigen or antibody is added, binding to the antigen-antibody complex.
4. **Substrate Addition:** A substrate is added that is converted by the enzyme into a detectable signal.
5. **Detection:** The resulting color change is measured, which is proportional to the concentration of the target antigen or antibody in the sample.

Quality Control Measures

ELISA Assay: The ApDia ELISA semi-autoanalyzer was used to AFP, HBsAg, HBeAg, Anti-HBe, Anti-HCV, and HIVp24 detection levels in plasma samples. Quality control measures were implemented to ensure the accuracy and reliability of the assay results. External positive and negative controls, provided by the manufacturer, were tested concurrently with each batch of plasma samples. These controls were essential for verifying the proper functioning of the test kits and ensuring that each assay run was valid. Additionally, calibration curves were generated using standard solutions, and the consistency of these curves was monitored across different assay batches.

Liver Function Tests: Liver function tests were conducted by spectrophotometric method using Biobase autoanalyzer (BS-230), which was calibrated regularly to maintain precision. The tests included measurements of ALT, AST, ALP, and bilirubin levels. Quality control was a critical component of the testing process, with both internal and external controls employed. The external controls, provided by the manufacturer, were tested alongside the plasma samples to verify the accuracy of the test kits and the reliability of the results. These controls were run with every batch to confirm the proper performance of the analyzer.

Quality Control Measures

To ensure the robustness of the data, stringent quality control measures were implemented throughout the

study. External positive and negative controls were run concurrently with each assay to verify the correct functioning of the analytical instruments and test kits. Additionally, calibration and internal control procedures were rigorously followed to minimize inter- and intra-assay variability.

Data Cleaning

Before proceeding with data analysis, all collected data underwent a thorough cleaning process. This step involved checking for any inconsistencies, outliers, or missing values that could affect the accuracy of the final results.

Statistical Analysis

The cleaned data were then analyzed using SPSS version 25. AFP levels were compared between groups using one-way ANOVA, followed by post hoc Tukey tests for pairwise comparisons. Pearson's correlation coefficient assessed correlations between AFP levels and clinical parameters. Temporal variations in AFP levels were analyzed using repeated measures ANOVA. A p-value <0.05 was considered statistically significant.

Results

The mean age of the participants was 42 ± 10 years, with a male-to-female ratio of 1.2:1. No significant differences were observed in age, gender, or socioeconomic status between the groups (Table 1).

Table 1. Sociodemographic Characteristics of Study Participants.

Characteristic	HBV Monoinfection (n=80)	HBV/HIV Co-infection (n=60)	HBV/HCV Co-infection (n=60)	p-value
Age (years)	42.3 ± 9.8	41.7 ± 10.2	42.8 ± 9.5	0.87
Gender (M/F)	44/36	32/28	33/27	0.72
Socioeconomic Status (Low/Medium/High)	34/30/16	25/23/12	28/22/10	0.81

AFP Levels and Liver Function Tests

AFP levels were significantly higher in the HBV/HCV co-infection group (36.2 ± 12.4 ng/mL) compared to the HBV monoinfection (12.5 ± 4.3 ng/mL) and HBV/HIV co-infection groups (18.7 ± 6.8 ng/mL) ($p<0.001$) (Table 2). Elevated liver function tests, particularly ALT and AST, were also more prevalent in the HBV/HCV co-infection group.

Table 2. AFP Levels and Liver Function Tests in Study Groups.

Parameter	HBV Monoinfection (n=80)	HBV/HIV Co-infection (n=60)	HBV/HCV Co-infection (n=60)	p-value
AFP (ng/mL)	12.5 ± 4.3	18.7 ± 6.8	36.2 ± 12.4	<0.001
ALT (U/L)	32.4 ± 10.2	45.7 ± 15.3	62.8 ± 20.1	<0.001
AST (U/L)	28.3 ± 9.5	40.2 ± 12.8	59.4 ± 18.6	<0.001
ALP (U/L)	110.7 ± 32.1	122.6 ± 38.4	135.2 ± 41.7	0.02
Bilirubin (mg/dL)	1.1 ± 0.3	1.4 ± 0.4	1.8 ± 0.6	<0.001

Correlation Analysis

AFP levels positively correlated with ALT ($r=0.52$, $p<0.01$) and AST ($r=0.47$, $p<0.01$) in the HBV/HCV co-infection group. No significant correlations were observed in the HBV monoinfection or HBV/HIV co-infection groups (Figure 1).

Figure 2 shows Alpha-Fetoprotein (AFP) levels measured over four quarters, (Q1 to Q4) for two groups of patients: those with both Hepatitis B and C (HBV/HCV co-infection) and those with only Hepatitis B (HBV monoinfection).

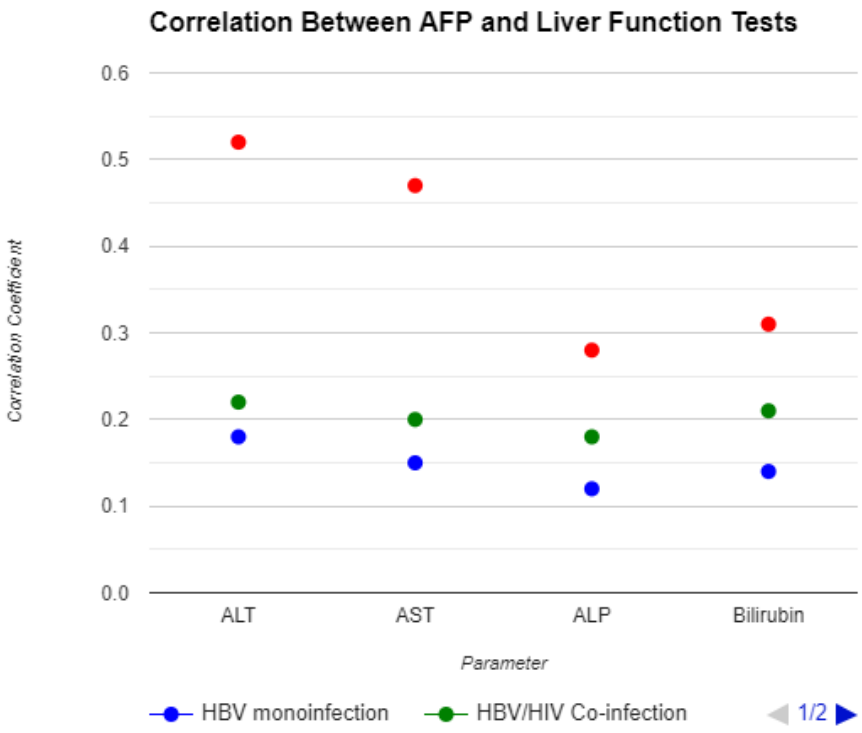

Figure 1. Correlation Analysis Between AFP and Liver Function Tests.

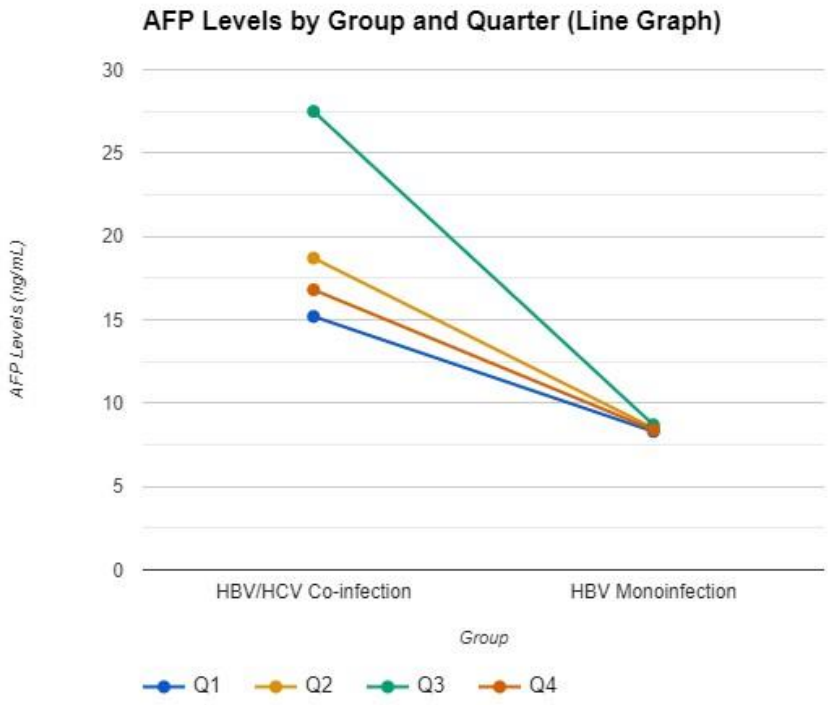

Figure 2. Quarterly Variations of Alpha Fetoprotein Levels in HBV/HCV Co-infection and HBV Monoinfection Groups among Subjects in Warri.

Discussion

This study evaluated Alpha-Fetoprotein (AFP) levels in HBsAg and HBeAg seropositive patients with and without HIV and HCV co-infections in Warri, Delta State, Nigeria, over one year. Our findings indicate that AFP levels are significantly higher in patients with HBV/HCV co-infection compared to those with HBV monoinfection or HBV/HIV co-infection. These elevated AFP levels in the HBV/HCV group suggest a heightened risk of liver disease progression and potential hepatocellular carcinoma (HCC) development. This observation aligns with previous studies that identified HCV co-infection as a factor that exacerbates liver disease in HBV patients (20-23).

The elevated AFP levels observed in HBV/HCV co-infected patients compared to those with HBV monoinfection or HBV/HIV co-infection underscore the additive or synergistic hepatocellular damage inflicted by HCV. HCV is known for its direct cytopathic effects and its ability to exacerbate liver inflammation, leading to more significant liver injury and, consequently, higher AFP levels. This is consistent with previous studies highlighting the exacerbation of liver disease in the presence of HCV, which may explain the pronounced increase in AFP levels observed in our study (24-27).

In contrast, the relatively modest increase in AFP levels among HBV/HIV co-infected patients suggests a different interaction between HIV and HBV in the liver. Although HIV is associated with chronic immune activation and inflammation, its direct impact on hepatocytes may be less pronounced compared to HCV. The immunosuppressive nature of HIV may also modulate the inflammatory response in a manner that does not significantly elevate AFP levels, despite the ongoing liver damage (28-31).

It is also possible that the antiretroviral therapy (ART) used in HIV-infected individuals plays a role in mitigating liver injury and, by extension, AFP production. ART has been shown to reduce HIV viral load and associated immune activation, potentially attenuating the extent of liver damage and AFP elevation in HBV/HIV co-infected individuals (23). However, the hepatotoxic potential of certain ART drugs cannot be ignored, and further research is needed to disentangle these complex interactions (24).

Another factor to consider is the differential immune response elicited by HCV and HIV in co-infected patients. HCV's ability to induce a more robust and sustained inflammatory response in the liver, as opposed to the more systemic immune dysregulation seen in HIV infection, might explain the observed differences in AFP levels. HCV's propensity to cause chronic liver inflammation and fibrosis may lead to increased AFP production as a marker of ongoing liver regeneration and damage (32-38).

Moreover, the role of AFP as a biomarker in these co-infection settings is multifaceted. While elevated AFP is a well-known marker for hepatocellular carcinoma (HCC), its utility in monitoring chronic liver disease progression, especially in co-infected patients, remains an area of active investigation (39-41). The correlation between AFP levels and liver function tests (LFTs) observed in this study further supports its potential role in tracking liver disease severity, particularly in HBV/HCV co-infection (26, 27).

However, the study also highlights the limitations of AFP as a sole biomarker, particularly in distinguishing between benign and malignant liver conditions in co-infected individuals. The modest correlations between AFP and LFTs in the HBV/HIV co-infected group suggest that AFP alone may not be sufficient to fully capture the complexity of liver disease in these patients (28, 29). This finding aligns with existing literature, which advocates for the use of a combination of biomarkers and imaging techniques for a more comprehensive assessment of liver health in co-infected individuals (30).

Our study emphasizes the need for a nuanced understanding of AFP dynamics in HBV co-infection contexts. The differential impact of HIV and HCV on AFP levels reflects the underlying pathophysiological differences in how these viruses interact with HBV and affect liver health. Future research should focus on elucidating the specific mechanisms through which HIV and HCV modulate AFP production and exploring the potential of AFP in combination with other biomarkers for improved clinical management of co-infected patients (42-44).

Limitation

This study has several limitations that should be considered. Firstly, the study population was limited to patients from Warri, Delta State, Nigeria, which may affect the generalizability of the findings to other regions with different demographics or healthcare settings. Additionally, the study's reliance on quarterly blood sample collections may have missed fluctuations in AFP levels occurring between these intervals. The exclusion of individuals with pre-existing liver disease or HCC may also limit the applicability of the results to patients with more advanced liver conditions. Lastly, the study did not account for potential variations in treatment regimens or adherence among participants, which could influence AFP levels and liver function outcomes

Conclusion

The study demonstrates that AFP levels are significantly higher in HBV patients with HCV co-infection compared to those with HBV monoinfection or HBV/HIV co-infection. This elevation in AFP suggests an increased risk of liver disease progression and potential hepatocellular carcinoma (HCC) in the HBV/HCV co-infection group. The observed positive correlations between AFP levels and liver enzymes (ALT and AST) in the HBV/HCV group further indicate ongoing liver damage and regeneration. These findings highlight the need for vigilant monitoring and management of HBV patients with HCV co-infection to address the heightened risk of liver complications.

Recommendations

1. Enhanced Monitoring: Routine AFP and liver function tests should be integrated into the care plans for HBV patients, particularly those with HCV co-infection, to facilitate early detection of liver disease progression and HCC.

2. Differentiated Management Strategies: Tailor treatment strategies based on co-infection status, with a focus on more aggressive monitoring for HBV/HCV co-infected patients. For HBV/HIV co-infected patients, emphasize maintaining immune function and monitoring liver health through regular assessments.

3. Public Health Initiatives: Strengthen public health programs to enhance awareness about the risks of co-infections and promote preventive measures, such as vaccination against HBV and harm reduction strategies to prevent HCV transmission. Implementing comprehensive screening programs can aid in early identification and intervention, improving patient outcomes.

Further Research: Future studies should explore the impact of various treatment regimens and adherence on AFP levels and liver disease progression. Expanding research to diverse populations and healthcare settings will help to validate and generalize the findings.

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Author contribution

MFO conceived the study, and participated in manuscript review, and overall research supervision. **KFA** participated in research design, data analysis, and manuscript writing. **ORO** participated in research design, data collation and manuscript writing. **TBO** participated in research design and sample collection. **TAM** participated in sample collection, data collation and manuscript writing. **AWT** participated in data analysis and manuscript review. **PNK** participated in research design and data analysis. **OJA** participated in research design and research supervision. **OBO** participated in sample analysis, data analysis, data collation and manuscript writing.

Conflict of interest

The authors declare that they have no competing interests.

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References

1. Hsu YC, Huang DQ, Nguyen MH. Global burden of hepatitis B virus: current status, missed opportunities and a call for action. Nat Rev Gastroenterol Hepatol. 2023;20(7):524–537.

2. Ajuwon BI, Yujuico I, Roper K, et al. Hepatitis B virus infection in Nigeria: a systematic review and meta-analysis of data published between 2010 and 2019. BMC Infect Dis. 2021;21:1120.

3. Hanif H, Ali MJ, Susheela AT, et al. Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. World J Gastroenterol. 2022;28(2):216-229.

4. Adigun OO, Yarrarapu SNS, Zubair M, et al. Alpha-fetoprotein analysis. StatPearls (Internet). 2024 Jan- (Updated 2024 May 1).

5. Głowska-Ciemny J, Szymański M, Kuszerska A, et al. The role of alpha-fetoprotein (AFP) in contemporary oncology: the path from a diagnostic biomarker to an anticancer drug. Int J Mol Sci. 2023;24(3):2539.

6. Zhu AX, Finn RS, Kang YK, et al. Serum alpha-fetoprotein and clinical outcomes in patients with advanced hepatocellular carcinoma treated with ramucirumab. Br J Cancer. 2021;124(8):1388–1397.

7. Tan A, Koh S, Bertoletti A. Immune response in hepatitis B virus infection. Cold Spring Harb Perspect Med. 2015;5(8):a021428.

8. Khanam A, Chua JV, Kottilil S. Immunopathology of chronic hepatitis B infection: role of innate and adaptive immune response in disease progression. Int J Mol Sci. 2021;22(11):5497.

9. Zhao HJ, Hu YF, Han QJ, Zhang J. Innate and adaptive immune escape mechanisms of hepatitis B virus. World J Gastroenterol. 2022;28(9):881-896.

10. Maini MK, Burton AR. Restoring, releasing or replacing adaptive immunity in chronic hepatitis B. Nat Rev Gastroenterol Hepatol. 2019;16(10):662–675.

11. Revill PA, Tu T, Netter HJ, et al. The evolution and clinical impact of hepatitis B virus genome diversity. Nat Rev Gastroenterol Hepatol. 2020;17(11):618–634.

12. Cheng Z, Lin P, Cheng N. HBV/HIV coinfection: impact on the development and clinical treatment of liver diseases. Front Med (Lausanne). 2021;8:713981.

13. Ruta S, Grecu L, Iacob D, Cernescu C, Sultana C. HIV-HBV coinfection-current challenges for virologic monitoring. Biomedicines. 2023;11(5):1306.

14. Weldemhret L. Epidemiology and challenges of HBV/HIV co-infection amongst HIV-infected patients in endemic areas: review. HIV AIDS (Auckl). 2021;13:485-490.

15. Hanif H, Ali MJ, Susheela AT, et al. Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. World J Gastroenterol. 2022;28(2):216-229.

16. Zhu AX, Finn RS, Kang YK, et al. Serum alpha-fetoprotein and clinical outcomes in patients with advanced hepatocellular carcinoma treated with ramucirumab. Br J Cancer. 2021;124(8):1388–1397.

17. Jasirwan COM, Fahira A, Siregar L, et al. The alpha-fetoprotein serum is still reliable as a biomarker for the surveillance of hepatocellular carcinoma in Indonesia. BMC Gastroenterol. 2020;20:215.

18. Cookey IT, Odenigbo KC, Okonkwo BJ, Okonko IO. Prevalence of HBsAg among patients attending a tertiary hospital in Port Harcourt, Nigeria. Int J Life Sci Res Arch. 2022;03(20):125-134.

19. Shea L, Pesa J, Geonnotti G, et al. Improving diversity in study participation: patient perspectives on barriers, racial differences and the role of communities. Health Expect. 2022;25(4):1979-1987.

20. Akuta N, Suzuki F, Kobayashi M, et al. Correlation between hepatitis B virus surface antigen level and alpha-fetoprotein in patients free of hepatocellular carcinoma or severe hepatitis. J Med Virol. 2014;86(1):131-8.

21. Hanif H, Ali MJ, Susheela AT, et al. Update on the applications and limitations of alpha-fetoprotein for hepatocellular carcinoma. World J Gastroenterol. 2022;28(2):216-229.

22. Bai DS, Zhang C, Chen P, et al. The prognostic correlation of AFP level at diagnosis with pathological grade, progression, and survival of patients with hepatocellular carcinoma. Sci Rep. 2017;7:12870.

23. Lin K, Huang Q, Zeng J, et al. Clinical significance of alpha-fetoprotein in alpha-fetoprotein negative hepatocellular carcinoma underwent curative resection. Dig Dis Sci. 2021;66(12):4545–4556.

24. Mavilia MG, Wu GY. HBV-HCV coinfection: viral interactions, management, and viral reactivation. J Clin Transl Hepatol. 2018;6(3):296-305.

25. Konstantinou D, Deutsch M. The spectrum of HBV/HCV coinfection: epidemiology, clinical characteristics, viral interactions and management. Ann Gastroenterol. 2015;28(2):221-228.

26. Desikan P, Rangnekar A, Khan Z, et al. Sero-occurrence of HBV/HCV co-infection and levels of liver enzymes among patients at a tertiary care hospital in central India: a pilot study. Cent Asian J Glob Health. 2019;8(1):313.

27. Tseng CW, Liu WC, Chen CY, et al. Impact of HCV viremia on HBV biomarkers in patients coinfected with HBV and HCV. BMC Infect Dis. 2022;22:351.

28. Sherman KE, Thomas DL. HIV and liver disease: a comprehensive update. Top Antivir Med. 2022;30(4):547-558.

29. Price JC, Thio CL. Liver disease in the HIV-infected individual. *Clin Gastroenterol Hepatol*. 2010;8(12):1002-12.

30. Xiao J, Zhang Y, Wu J, et al. HIV/HBV coinfection: understanding the complex interactions and their impact on spontaneous HBV clearance, chronic liver damage, cirrhosis, and hepatocellular carcinoma. AIDS Rev. 2024;26(1):32-40.

31. Chamroonkul N, Bansal MB. HIV and the liver. Nat Rev Gastroenterol Hepatol. 2019;16(1):1–2.

32. Gopalakrishna H, Mironova M, Dahari H, et al. Advances and challenges in managing hepatitis D virus: evolving strategies. Curr Hepatol Rep. 2024;23:32–44.

33. Klatte K, Pauli-Magnus C, Love SB, et al. Monitoring strategies for clinical intervention studies. Cochrane Database Syst Rev. 2021;12(12):MR000051.

34. Goorts K, Dizon J, Milanese S. The effectiveness of implementation strategies for promoting evidence-informed interventions in allied healthcare: a systematic review. BMC Health Serv Res. 2021;21(1):241.

35. Carr BI. Overview of clinical HCC and its management. Liver Cancer Middle East. 2021;7:123–134.

36. Fanning GC, Zoulim F, Hou J, et al. Therapeutic strategies for hepatitis B virus infection: towards a cure. Nat Rev Drug Discov. 2019;18(11):827–844.

37. Klaic M, Kapp S, Hudson P, et al. Implementability of healthcare interventions: an overview of reviews and development of a conceptual framework. Implement Sci. 2022;17(10).

38. Ricciardi W, Cascini F. Guidelines and Safety Practices for Improving Patient Safety. 2020 Dec 15. In: Donaldson L, Ricciardi W, Sheridan S, et al., editors. Textbook of Patient Safety and Clinical Risk Management (Internet). Cham (CH): Springer; 2021. Chapter 1.

39. Bolton RE, Bokhour BG, Hogan TP, Luger TM, Ruben M, Fix GM. Integrating Personalized Care Planning into Primary Care: a Multiple-Case Study of Early Adopting Patient-Centered Medical Homes. J Gen Intern Med. 2020;35(2):428-436

40. Goetz LH, Schork NJ. Personalized medicine: motivation, challenges, and progress. Fertil Steril. 2018 Jun;109(6):952-963.

41. Simmons LA, Drake CD, Gaudet TW, Snyderman R. Personalized Health Planning in Primary Care Settings. Fed Pract. 2016 Jan;33(1):27-34.

42. Shahzad, M., Upshur, R., Donnelly, P. et al. A population-based approach to integrated healthcare delivery: a scoping review of clinical care and public health collaboration. BMC Public Health 19, 708 (2019).

43. Windle M, Lee HD, Cherng ST, Lesko CR, Hanrahan C, Jackson JW, McAdams-DeMarco M, Ehrhardt S, Baral SD, D'Souza G, Dowdy DW. From Epidemiologic Knowledge to Improved Health: A Vision for Translational Epidemiology. Am J Epidemiol. 2019 Dec 31;188(12):2049-2060.

44. Trent RJ. Public Health, Communicable Diseases and Global Health. Molecular Medicine. 2012:169–201.

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Case report Free Access

Ecthyma gangrenosum with a coinfection of methicillin-sensitive staphylococcus aureus and streptococcus pyogenes: a case report

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Abstract

Introduction: Ecthyma gangrenosum (EG) is a cutaneous infection characterized by gangrenous ulcers with erythematous borders seen in immunocompromised as well as immunocompetent individuals. Although *Pseudomonas aeruginosa* is the commonest pathogen isolated, several other bacteria and fungi contribute to the pathogenesis of EG. Identification of the microorganism is very essential to initiate early empirical antimicrobial therapy.

Case presentation: We present a case report of a 13-year-old boy with multiple recurrent ulcerative lesions in both lower extremities for the past 1 year. His blood parameters showed signs of inflammation but was negative for aerobic blood culture, suggesting absence of underlying bacteraemia. There were no features of immunosuppression. On examination of pus sample, Methicillin Sensitive *Staphylococcus aureus* and *Streptococcus pyogenes* were isolated from the ulcerative lesions. Amoxicillin- Clavulanate and Doxycycline was advised for 2 weeks along with surgical debridement of the lesion followed by aseptic dressing. Patient showed complete resolution after 2 weeks.

Discussion: *Staphylococcus aureus* and *Streptococcus pyogenes* were the causative agents in this case, suggesting a polymicrobial association of EG besides *Pseudomonas aeruginosa*. Underlying bacteraemia or any other immunodeficiency is usually seen in a case of EG, however there are cases reported where cutaneous manifestations show predominance.

Conclusion: A prompt diagnosis of EG is essential because there are instances when it has proven to be fatal. Ruling out any immunodeficiency disorders and underlying bacteraemia is of vital importance. Administration of proper antibiotic coverage (gram positive or gram negative) along with debridement and regular dressing can help in limiting the spread of infections and thus improving patient outcomes.

Keywords: Ecthyma, Staphylococcus aureus, Streptococcus pyogenes

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Introduction

Ecthyma gangrenosum (EG) is a cutaneous infection that causes crusted lesions beneath which ulcers develop. It has deeper dermal infiltration, leading to severe manifestations as compared to impetigo but both conditions have similar bacterial causative agents. EG occurs most commonly in immunocompromised individuals, however, healthy immunocompetent people are not always excluded. Common risk factors include neutropenia, leukemia, multiple myeloma, type 2 diabetes, malnutrition, and significant burn injury (1).

Gangrenous ulcers with erythematous borders generally characterize lesions. Primarily affecting the axillary and anogenital regions it can also involve the arms, legs, trunk, and face. The characteristic macroscopic appearance is caused by perivascular invasion and ischemic necrosis of the associated skin (1).

Pseudomonas aeruginosa is the most common bacteria found in EG. *P. aeruginosa* infection is rare in healthy children, but could occur in patients with croup syndrome and sepsis. In fact, EG may be the first sign of a *Pseudomonas* infection or might even develop in the later course. It usually appears before the results of the blood culture and help clinicians to choose appropriate antibiotics. *Methicillin-resistant Staphylococcus aureus* (MRSA), *Streptococcus pyogenes, Citrobacter freundii, Escherichia coli, Aeromonas hydrophila, Serratia marcescens, Aspergillus spp., Mucor spp.,* *and Candida spp.* are among the many other causes of EG (2).

This report suggests that besides *Pseudomonas aeruginosa*, EG due to coinfection with other microorganisms, such as *Staphylococcus aureus* and *Streptococcus pyogenes* even though rare, can prove to be a significant finding, especially in the absence of bacteraemia or any other immunocompromised status. Hence, prompt diagnosis with early initiation of appropriate antibiotics can prevent further complications and fatalities.

Case presentation

We present the case of a 13-year-old boy with complaints of multiple recurrent ulcerative lesions in both lower extremities for the past 1 year. The lesions were itchy and slightly painful. Throughout the past year, on application of topical ointments, there was temporary remission of lesions which later flared up. There was no history of any insect bite. Local examination revealed that the lesions were in varied stages of development. Some exhibited pustules, while others had punched-out ulcers with thick, brown-black crusts and surrounding erythema (Figure 1). His physical examination revealed mild anaemia but no local lymphadenopathy.

Figure 1. Multiple punched-out ulcerative lesions with thick, brown-black crusts and surrounding erythema over lower extremities.

His blood parameters revealed mildly raised WBC count of 13000/µL (reference value: 4000–11,000/µL), ESR 55 mm/hr (reference value: 0-15 mm/hr), CRP 150 mg/dl (reference value: ≤0.8 mg/dL), and procalcitonin 6.25 ng/ml (reference value: ≤0.10 ng/mL). (All reference values were taken from American Board of Internal Medicine Laboratory Test Reference Ranges− July 2023). All serological parameters were negative. Aerobic Blood culture was negative after 5 days of incubation in BD BACTEC™ FX40.

Skin biopsy was taken as well as pus collected from underneath the crusts. Gram stain of the pus revealed plenty of pus cells with gram-positive cocci in chains as well as in clusters. Ziehl-Neelsen staining with 20%

H_2SO_4 was negative for acid fast bacilli. Two cultures were done on Blood agar, one incubated aerobically at 37˚ C, and the other incubated in the presence of 10% CO_2. After overnight incubation, *Staphylococcus aureus* and *Streptococcus pyogenes* were isolated. Antibiotic susceptibility testing was performed by Modified Kirby Bauer Disc Diffusion method on Mueller Hilton agar for *Staphylococcus aureus* and Mueller Hilton agar with 5% Sheep blood for *Streptococcus pyogenes* as per CLSI 2023 guidelines. Zone diameters were measured (3). *Staphylococcus aureus* was Penicillin and Clindamycin resistant, intermediate susceptible to Ciprofloxacin and susceptible to Linezolid, Erythromycin, Cefoxitin, Doxycycline and Cotrimoxazole. *Streptococcus pyogenes* was resistant to Clindamycin but susceptible to penicillin, erythromycin, and linezolid. Skin biopsy revealed inflammatory cell infiltration, vascular proliferation, extensive keratinocyte necrosis along with cocci in clusters and in chains. However, no bacilli, amastigote forms (Leishman Donovan bodies) or fungal hyphae were found.

The patient underwent debridement of the ecthyma crusts along with a 14 day oral course of Amoxycillin-Clavulanate (625 mg thrice daily) and Doxycycline (100 mg twice daily). On follow-up examination of the patient after 2 weeks, no new lesions were seen and there was resolution of the debrided ulcers. The patient was advised to maintain strict hygiene of the affected sites and his parents were counselled to ensure proper nutrition of the child.

Discussion

Few differential diagnoses of EG includes other causes of necrotic wounds such as, cutaneous anthrax, cutaneous aspergillosis, cutaneous leishmaniasis, *Mycobacterium marinum* infection and pyoderma gangrenosum (4). However, absence of bacilli, amastigote forms of Leishmaniasis or septate hyphae fungal in the pus sample as well as skin biopsy eliminates the first three differentials. Acid fast stain was negative for Mycobacterial infections and absence of any relevant underlying conditions, such as inflammatory bowel disease excludes pyoderma gangrenosum. EG is also often confused with Ecthyma contagiosum which is characterized by solitary pustular lesions on hands and results from the direct contact of damaged skin with animals infected by a virus of Parapoxvirus genus: Orf virus (5).

The diagnosis of EG is not excluded even if blood culture yields a negative result. Pus, tissue, and exudate cultures could be used for identifying the organism causing the lesion. When both cultures show negative results, histopathological examination and KOH mount should be performed.

EG is usually due to *Pseudomonas aeruginosa* bacteraemia in patients with impaired immune systems. However, patients without any underlying immunodeficiencies may also suffer from this clinical situation and even without any features of bacteraemia (6,7,8). This is highlighted in our case where EG occurred in an immunocompetent patient without bacteraemia and with causative organisms besides *Pseudomonas aeruginosa* as Coinfection with Methicillin Sensitive *Staphylococcus aureus* (MSSA) and *Streptococcus pyogenes* was seen in this case. Ecthyma gangrenosum secondary to MSSA was also seen in a case reported by Ivanaviciene J et al. (9).

Here, the *Staphylococcus aureus* strain was resistant to penicillin, whereas the beta-haemolytic *Streptococcus pyogenes* was susceptible. Oral combination antimicrobial therapy with Beta lactam-beta lactamase inhibitor (BL-BLI) and a broad-spectrum antibiotic was required to manage this condition. Kudo Nagata Y et al. reported cases of EG with MRSA strains, which could be fatal, especially in patients with haematological malignancies due to concurrent bacteraemia. Although such a case is relatively uncommon, tissue cultures with an initial gram stain is essential for selecting appropriate empirical antimicrobials, including the coverage of *S. aureus* (10). Ulpiano Trillig, A et al. also reported two cases of coinfection by group A *Streptococcus spp.* and *Staphylococcus aureus* admitted to the hospital. The first patient had no risk factors nor any immunodeficiency, but the second case was a homeless man with drug and alcohol abuse and advanced HIV infection (11). A study in Japan showed that staphylococcal infection was responsible for 60% of cases of EG, while the remaining cases were attributed to *Streptococcal* and *P. aeruginosa* infections, in descending order of prevalence (12).

There are even two postulated mechanisms identified in the literature that describe the pathogenesis of EG. In the first form, bacteria from a primary infection originating in the genitourinary, respiratory, or gastrointestinal tract travel hematogenously, disseminating through the vasculature to the skin, or in the second scenario a cutaneous abnormality emerges and microbial infiltration takes place at the precise location of the abnormality (13). Lesions usually recover after surgical debridement of the ulcers with a complete course of antibiotics. Maintenance of proper hygiene is also required to prevent recurrence.

Conclusion

Ecthyma gangrenosum is a serious and sometimes fatal skin condition that initially manifests as a maculopapular rash, followed by a haemorrhagic bulla, necrotic ulceration, and surrounding erythema. The perivascular bacterial invasion of cutaneous blood vessels resulting in ischemic skin necrosis is the main pathology behind EG. A clinical diagnosis is often established by punched-out ulcers with thick, brown-black crusts. Lesions might be one or more, and, as seen in our case, they can be in different phases of development. There are several bacterial agents responsible for this condition and thus it might sometimes be polymicrobial. Proper antibiotic therapy along with hygiene maintenance is essential to treat this skin condition.

Ethical consideration and consent

Ethical clearance was obtained from Institutional Ethical Committee. Informed written consent was obtained from the patient to publish this case report (MGM/PRI/GEM-86/2024).

Author contribution

RDR was responsible for conceptualization and writing the original draft. **DD** contributed to the methodology, supervision and reviewing the manuscript. **SDG** helped in writing and reviewing the original draft and data curation.

Conflict of interest

The authors declare that they have no competing interests.

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References

1. Vaiman M, Lazarovitch T, Heller L, Lotan G. Ecthyma gangrenosum and ecthyma-like lesions: review article. Eur J Clin Microbiol Infect Dis. 2015 Apr; 34(4): 633-39.

2. Fang LC, Peng CC, Chi H, Lee KS, Chiu NC. Pseudomonas aeruginosa sepsis with ecthyma gangrenosum and pseudomembranous pharyngolaryngitis in a 5-month-old boy. J Microbiol Immunol Infect. 2014 Apr; 47(2): 158-61.

3. CLSI. M100™ Performance Standards for Antimicrobial Susceptibility Testing, 33rd ed. CLSI supplement M100.Clinical Laboratory Standards Institute; 2023.

4. Ozkaya O, Uscetin I, Egemen O. Reconstructive procedure of lower lip defect due to ecthyma gangrenosum: a rare complication of acute lymphoblastic leukemia. J Craniofac Surg. 2012; 23: E182–184.

5. Mavridou K, Bakola M. Orf (ecthyma contagiosum). Pan Afr Med J. 2021; 38:322.

6.Koo SH, Lee JH, Shin H, Lee JI. Ecthyma gangrenosum in a previously healthy infant. Arch Plast Surg. 2012; 39: 673–75.

7. Yan W, Li W, Mu C, Wang L. Ecthyma gangrenosum and multiple nodules: Cutaneous manifestations of Pseudomonas aeruginosa sepsis in a previously healthy infant. Pediatr Dermatol. 2011; 28: 204–5.

8. Goolamali SI, Fogo A, Killian L, Shaikh H, Brathwaite N, Ford-Adams M, *et al*. Ecthyma gangrenosum: an important feature of pseudomonal sepsis in a previously well child. Clin Exp Dermatol. 2009 Jul; 34(5): E180-2.

9. Ivanaviciene J, Chirch L, Grant-Kels JM, Kerr PE, Finch J. Ecthyma gangrenosum secondary to

methicillin-sensitive *Staphylococcus aureus*. Int J Womens Dermatol. 2016 Jul 22; 2(3): 89-92.

10. Kudo Nagata Y, Sekiya N, Fukushima K, Horiuchi M, Doki N. Ecthyma gangrenosum caused by Staphylococcus aureus in hematological malignancies: Case reports and literature review. Medicine (Baltimore). 2022 Aug 19;101(33): E30070.

11. Ulpiano Trillig A, Miendje Deyi VY, Youatou P, Konopnicki D. Echtyma gangrenosum caused by coinfection with *group A Streptococcus* and *Staphylococcus aureus*: an emerging etiology? Case reports and literature review. Acta Clinica Belgica. 2019; *76*(1): 53–57.

12. Tomoaki I, Yoshihiko S, Maki T, Natsuko D. Ecthyma Gangrenosum-Like Lesions in a Healthy Child after Infection Treated with Antibiotics. Pediatr. Dermatol.2005; 22: 453-456.

13. Hajaj H, Bahari H, Zahiri H, Ghanam A, El Ouali A, et al. Ecthyma Gangrenosum in Patient with Bone Marrow Aplasia: A Case Report and Review of the Literature. Open J. Pediatr. 2024;14: 272-278.