CASE REPORT

CD20-Negative Secondary Breast Lymphoma Following Rituximab Therapy: A Case Report

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ABSTRACT

Breast lymphoma is an infrequent form of extranodal lymphoma, but it is the most common haematological malignancy involving the breast. It is categorized into primary and secondary forms, with the latter being more common. We present a case of a 72-year-old woman who was diagnosed with CD20-positive diffuse large B-cell lymphoma (DLBCL) of the cervical lymph nodes. During chemotherapy, she developed bilateral breast swelling. Needle core biopsies of the breast lumps confirmed DLBCL with loss of CD20 expression. It has been reported in the literature that CD20 negativity may occur following rituximab therapy and is frequently associated with poor outcomes. This case highlights the phenomenon of CD20 loss in secondary breast lymphoma following rituximab therapy, emphasizing the importance of a detailed clinical history, awareness of diagnostic pitfalls, and the need for further research into clinical implications and management.

Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP12):137-140. doi:10.47836/mjmhs.21.s12.26

Keywords: CD20 negative, Secondary breast lymphoma, Rituximab, B-cell lymphoma, Diffuse large B-cell lymphoma

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INTRODUCTION

Lymphoma is a common haematological malignancy; however, its involvement in the breast is rare, accounting <0.5% of all malignant neoplasm of breast (1). This rarity is likely due to the small amount of lymphoid tissue present within the breast. Primary breast lymphoma (PBL) is defined as lymphoma confined to unilateral or bilateral breast and/or regional lymph nodes in the absence of prior history of lymphoma (1). Based on criteria proposed by Wiseman and Liao, which include: (i) adequate pathological evaluation, (ii) the presence of mammary tissue with a lymphomatous infiltrate, (iii) no evidence of lymphoma at other sites, except for simultaneous lymph node involvement at same side, and (iv) no history of diagnosis of lymphoma, any breast lymphoma that does not meet these criteria is classified as secondary breast lymphoma (SBL) (2) and the most commonly reported secondary breast lymphoma is DLBCL, NOS (1). The common age of presentation in both primary and secondary breast lymphoma is between 40-67 years old with higher prevalence seen among female (2). It was reported that 11% of cases of primary breast lymphoma involved bilateral breast (1) while for secondary breast lymphoma, it typically presented as multiple breast lesion (2). Breast lymphomas, whether primary or secondary, has generally poor prognosis, with five-year survival rates ranging from 9% to 85%, depending on prognostic factors such as histologic subtype and the clinical stage at diagnosis based on the Ann Arbor system (2). The R-CHOP protocol, which consists of rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone, is a well-established treatment option for DLBCL (3,5). Although CD20 antigen loss following rituximab therapy is a known phenomenon in relapsed

or refractory B-cell lymphomas, reported cases involving secondary breast lymphoma are exceedingly rare. This case report highlights the rarity of such presentation and aims to raise awareness of this diagnostic pitfall. We summarize the potential mechanisms underlying CD20 antigen loss, emphasize the diagnostic utility of alternative B-cell lineage markers such as PAX5 and CD79a, reinforce the need of repeating biopsy in relapsed case and discuss the prognosis and therapeutic challenges associated with CD20-negative relapsed disease.

CASE REPORT

A 72-year-old female with underlying hypertension, diabetes mellitus, right middle cerebral artery infarct, and atrial flutter presented with cervical lymphadenopathy and weight loss in late 2023. An excision biopsy of a left level V lymph node was performed in December 2023, and histopathological examination revealed diffuse sheets of medium to large atypical lymphoid cells that exhibited pleomorphic vesicular nuclei with prominent single to multiple nucleoli (Fig 2a). Immunohistochemistry (IHC) showed positivity for CD20 (Fig 2b), CD79a, PAX5, CD10, and BCL-6, with a Ki67 proliferative index of 80%. Based on these findings, the patient was diagnosed with DLBCL, germinal centre subtype, of the cervical lymph node. Chemotherapy includes rituximab was initiated in February 2024 with a monthly cycle interval.. During her admission for fifth cycle of chemotherapy in June 2024 which is approximately one month after the last cycle, , she complained of bilateral breast swelling. Breast ultrasound was immediately performed and revealed ill-defined mass-like lesions in both breasts, with intralesional vascularity and a few subcentimeter axillary lymph nodes. Needle core biopsies of the right and left breast lesions were performed in July 2024 (seven months since the initial diagnosis of DLBCL of cervical lymph node) and the histopathological examination showed fibrofatty tissue diffusely infiltrated by neoplastic lymphoid cells with histomorphology features similar to that of the previously biopsied lymph node (Fig 3a). However, the immunohistochemistry (IHC) of the breast biopsy showed negative for CD20 (Fig 3b) but remained immunoreactive for CD79a (Fig 3c) and PAX5 (Fig 3d). The Ki67 proliferative index was approximately 95%. The bilateral breast lesions were diagnosed as high-grade B-cell lymphoma, likely refractory DLBCL. Given the patient's advanced age, frailty, and lack of response to first-line chemotherapy, she was referred for palliative care and she was lost to follow-up after her first palliative clinic visit in August 2024. Fig 1 provides a summary of the clinical timeline of the case.

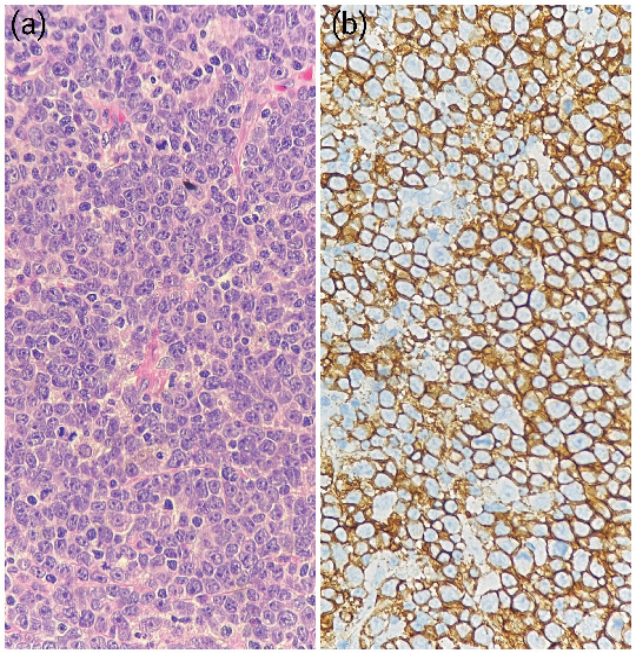

Fig 2: Lymph node: Haematoxylin and eosin (H&E) stain ×400 (a) showed diffuse sheets of medium to large atypical lymphoid cells. Immunohistochemical (IHC) stain ×400 CD20 (b) showed diffuse strong membranous positivity for CD20.

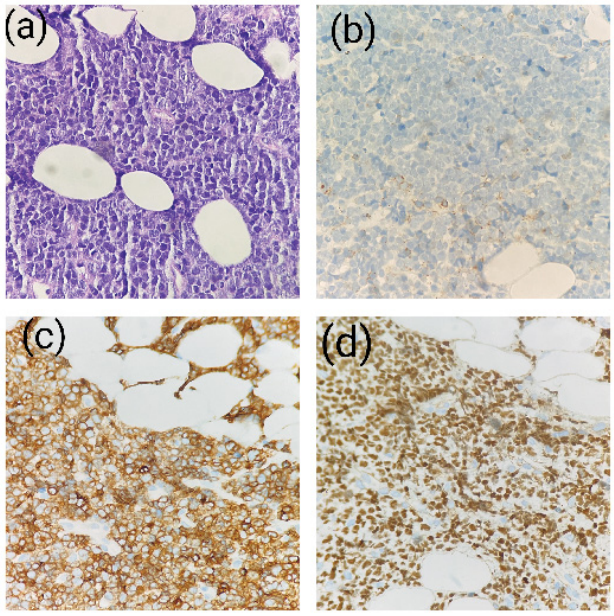

Fig 3: Breast: Haematoxylin and eosin (H&E) stain ×400 (a) showed similar morphology in needle core biopsy of breast lesion with previous cervical lymph node biopsy specimen. Immunohistochemical (IHC) stain ×400 CD20 (b) showed negative staining. CD79a (c) showed diffuse membranous positivity and PAX5 (d) showed strong nuclear positivity.

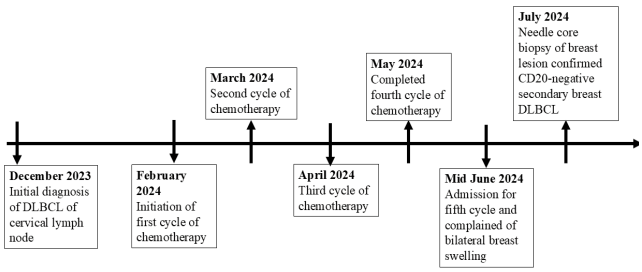

Fig 1: Summary of the clinical timeline of the case.

DISCUSSION

The CD20 molecule is a transmembrane, non-glycosylated phosphoprotein expressed on the surface of all mature B lymphocytes and neoplastic B cells (3,5). It is encoded by MS4A1, a member of the membrane-spanning 4-domain family, subfamily A (MS4A).

Rituximab, a chimeric monoclonal antibody targeting the CD20 B-cell antigen, has become a cornerstone in the treatment of B-cell lymphomas, including DLBCL, when combined with CHOP chemotherapy (3). It selectively targets CD20-positive B cells through several mechanisms including complement mediated cytotoxicity, antibody-dependent cellular cytotoxicity and induction of apoptotic pathway (5).

A recent study by Marshalek et al. reported an 11.9% rate of CD20 expression loss among 59 patients with prior CD20-positive B-cell lymphoma, all of whom had received rituximab-based therapy (4). In the same study, most of the relapsed cases involves lymph nodes (4). In our literature review, no definitive prevalence data were found for CD20-negative secondary breast lymphomas post-rituximab highlighted the novelty of this case.

Two primary mechanisms have been proposed: (i) selective elimination of CD20-positive cells which promotes the proliferation of CD20-negative cells, and (ii) direct induction of CD20 loss in previously CD20-positive clones through gene mutation and reduced transcription (4-5). Wada et al. demonstrated the presence of genetic instability in post-rituximab cases (5). This study also highlighted that deletional mutations at the C-terminus of the CD20 molecule and reduced transcription, possibly due to impaired regulatory protein binding at the CD20 gene promoter, contribute to the progressive loss of CD20 expression with repeated rituximab exposure (5). CD20 negative lymphoma are known to be resistant to rituximab therapy, and such cases are often associated with treatment resistance and progressive disease leading to a poor prognosis (5). Therefore, considering the potential for antigenic modulation following rituximab therapy, a repeat biopsy is essential in cases of relapsed or secondary lymphoma—such as in our case—to reassess the immunophenotypic profile and to guide in therapeutic approach.

CD20-negative lymphomas pose significant challenges in both diagnosis and treatment. In terms of diagnosis, CD20 is a widely used B-cell lineage marker and is often used as an initial single B-cell markers, especially in resource-limited setting in diagnosing and subtyping lymphoma. CD20 negativity can lead to misdiagnosis, particularly if a history of prior rituximab therapy is not properly elicited.

Alternative B-cell lineage markers, such as PAX5, CD79a, OCT2, and BOB1, should be utilized for immunophenotyping CD20-negative B cell lymphomas, especially in cases of recurrent B-cell lymphomas when the tissue biopsy specimen is obtained during rituximab therapy for previous CD20-positive lymphoma (3). PAX5, a member of the paired box (PAX) family of transcription factors, encodes a B-cell specific activator protein that is present in B cells, making it a reliable B-cell lineage marker (3). On the other hand, the B-cell receptor consists of surface immunoglobulin and CD79a/b. Therefore, CD79a, which is exclusively found on B cells and B-cell neoplasms, is also a useful marker for B cells. The application of immunohistochemistry of PAX5 and CD79a in our case further supports the existing literature regarding their utility as alternative B-cell markers, particularly in CD20-negative lymphoma. Furthermore, OCT2 and BOB1 are now recognized as comprehensive B-lineage immunohistochemical markers. These transcription coactivators, specific to B lymphocytes, play vital roles in the development of the germinal centre and in the synthesis of immunoglobulins (3). In HIV-associated lymphomas, which are typically negative for routine B cell markers, previous studies have reported that OCT2 and BOB1 may serve as useful B lineage markers for diagnosing these rare types of lymphoma (3). The use of a second B-cell marker is essential in the immunophenotyping of lymphoma, particularly in cases where CD20 expression is absent. Relying solely on CD20 for lineage confirmation can lead to diagnostic ambiguity and may result in unnecessary or misdirected ancillary testing. This is especially relevant in relapsed or post-treatment cases. Employing additional B-cell markers, such as PAX5, CD79a, OCT2 and BOB1, helps ensure accurate lineage assignment and prevents diagnostic and therapeutic missteps.

Loss of CD20 expression has significant therapeutic implications, as CD20-directed therapies, which constitute a cornerstone of standard treatment, become ineffective in the absence of target antigen expression. There are 40% to 60% of cases reported in which rituximab is initially effective but fails upon retreatment and this resistance may be attributed to the loss of CD20 expression in the neoplastic B cells (3). Currently, there is no defined therapeutic protocol for CD20-negative B-cell lymphoma. Salvage cytotoxic chemotherapy such as ICE (ifosfamide, carboplatin and etoposide) and DHAP (dexamethasone, cytarabine and cisplatin) have been used for treatment (4). Additionally, novel therapeutic strategies like CAR T-cell therapy have been explored (4). However, the clinical data on these strategies is limited and hence further research on CD20 loss and its management is warranted.

A key limitation of this case report is the absence of additional molecular studies, particularly fluorescence in situ hybridization (FISH) testing for MYC and BCL2 and/or BCL6 gene rearrangements, which are essential for excluding double-hit or triple-hit high-grade B-cell lymphoma—a subtype known to exhibit resistance to

standard R-CHOP chemotherapy. The lack of these molecular data limits the ability to fully characterize the tumour's biological behaviour and to guide optimal therapeutic strategies.

CONCLUSION

Although breast lymphoma is rare, it should be considered in patients presenting with multiple breast lesions, especially when there is prior history of lymphoma elsewhere. A history of rituximab treatment is particularly important when approaching a case of recurrent lymphoma, as rituximab may cause CD20-negativity and result in misdiagnosis if it is not properly elicited. In such cases, alternative immunohistochemistry panel such as CD79a, PAX5, OCT2, BOB1 should be used for immunophenotyping. In summary, a combination of thorough clinical history, histomorphological features with reliable immunohistochemical results are essential to improve the diagnosis of CD20-negative B cell lymphomas. Further research is warranted for understanding its mechanism and management of these challenging cases.

ACKNOWLEDGEMENT

The authors acknowledge the Histopathology Unit, Department of Pathology, Hospital Raja Permaisuri Bainun, for providing this case and for their assistance with the immunohistochemical studies, which were essential in characterizing this rare breast pathology.

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