GASTROINTESTINAL TRACT PLASMA BLASTIC LYMPHOMA

IN

HIV INFECTED ADULTS:

A HISTOPATHOLOGICAL AUDIT

by

ABSALOM MWAZHA
Student Number: 214584095

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School of Laboratory Medicine and Medical Sciences

Nelson R Mandela School of Medicine

University of KwaZulu-Natal

Durban

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DEDICATION

This work is dedicated with gratitude to my father Runokovangwa Corneus Mwazha and to my mentors.
I am truly grateful to my supervisor Dr GB Nhlonzi for his invaluable assistance, guidance, patience and trust in my abilities throughout this project.

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My heartfelt thanks are due to all my friends and family for their patience, support and helping to keep me sane.
ABSTRACT

Background: Plasmablastic lymphoma (PBL) is an aggressive B-cell lymphoma that is characterised by the expression of plasma cell antigens and loss of pan B-cell antigens. The neoplasm is extensively reported in the oral cavity and anorectal region but rarely in the gastrointestinal tract where only isolated case reports and small case series exist. In the current study, morphologic, immunohistochemical and molecular features of 17 cases of gastrointestinal tract PBL were reviewed.

Materials and Methods: Ten-year retrospective study that reappraised the histomorphological and immunophenotypic features of HIV-associated PBLs in the gastrointestinal tract that were diagnosed and coded as ‘plasmablastic lymphoma’.

Results: The average age of the study patients was 41 years with a 3:1 ratio of males to females. The most frequent site of involvement was the small intestine (42%). Majority of the cases showed a predominant diffuse (82%) growth pattern. Immunoblasts and plasmablasts were observed in all cases. Sixty-five percent (65%) of the cases exhibited scattered centroblasts and one case demonstrated predominance of centroblasts. Other features observed include pseudo-alveolar growth pattern, plasmacytic differentiation, scattered multinucleated giant cells, focal clear cell change, high mitotic activity with high proliferative indices (Ki-67 >90%), apoptotic bodies and necrosis. Immunohistochemistry revealed absence of pan B-cell antigens and expression of plasma cell antigens. Epstein-Barr virus-encoded RNA was expressed in 53% of the cases.

Conclusion: This study highlights the spectrum of histopathological features of gastrointestinal tract PBLs. Additional observations not previously described or emphasised in literature includes pseudo-alveolar growth pattern, centroblast-predominance, multinucleated giant cells and clear cell change. Awareness of this entity in the gastrointestinal tract and its histopathological features and immunohistochemical profile is essential for making an accurate diagnosis and avoiding potential diagnostic errors.

Keywords: Plasmablastic lymphoma; HIV-related lymphoma; AIDS-related lymphoma; gastrointestinal tract; stomach; small intestine; colon
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LIST OF ABBREVIATIONS

A20............................... Anti-inflammatory protein 20
AIDS.............................. Acquired immune deficiency syndrome
ALK................................ Anaplastic lymphoma kinase
ALK LBL.......................... Anaplastic lymphoma kinase positive large B-cell lymphoma
BARTs......................... BamH1 A rightward
BCL-2............................. B-cell lymphoma 2
BCL-6............................. B-cell lymphoma 6
BL................................. Burkitt lymphoma
CD................................. Cluster of differentiation
DAPI.............................. 4’, 6-diamidino-2-phenylindole
DLBCL.......................... Diffuse large B-cell lymphoma
DNA.............................. Deoxyribonucleic acid
EBER............................ Epstein–Barr virus-encoded small RNA
EBNA............................ Epstein–Barr virus nuclear antigen
EBV.............................. Epstein–Barr virus
EPBL............................. Extra-oral plasmablastic lymphoma
EMA.............................. Epithelial Membrane Antigen
FFPET............................ Formalin-fixed paraffin-embedded tissue
FISH.............................. Fluorescence in-situ hybridisation
HAART.......................... Highly active antiretroviral treatment
H&E............................... Haematoxylin and eosin
HIV.............................. Human immunodeficiency virus
HHV8............................ Human herpes virus 8
IALCH..........................Inkosi Albert Luthuli Central Hospital

IgH...............................Immunoglobulin heavy chain

IHC.................................Immunohistochemistry

IL-6.................................Interleukin 6

IRF4.................................Interferon regulatory Factor-4

ISH.................................In-situ hybridisation

KSHV...............................Kaposi sarcoma-associated herpesvirus

LCA.................................Leucocyte common antigen

LMP.................................Latent membrane protein

LP.................................Leader protein

MALT1.............................Mucosa-associated lymphoid tissue lymphoma translocation protein 1

MM.................................Multiple myeloma

MUM-1.............................Multiple myeloma oncogene-1

MYC.................................Myelocytomatosis Viral Oncogene Homolog

ND.................................Not done

NHL.................................Non-Hodgkin lymphoma

NHLS..............................National Health Laboratory Service

PAX5...............................Paired box protein 5

PBL.................................Plasmablastic lymphoma

PCNSL.............................Primary central nervous system lymphoma

PCR.................................Polymerase chain reaction

PEL.................................Primary effusion lymphoma

PRDM1.............................PR domain zinc finger protein 1

PTCL...............................Peripheral T-cell Lymphoma
1. INTRODUCTION

Lymphomas which arise in human immunodeficiency virus (HIV) positive patients are heterogeneous and include those which are usually diagnosed in immunocompetent patients as well as those that are commonly seen in the setting of HIV infection [1]. The HIV-associated lymphomas commonly encountered include Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL), primary effusion lymphoma (PEL) and plasmablastic lymphoma (PBL) [1].

Plasmablastic lymphoma (PBL) is an aggressive high-grade B-cell non-Hodgkin lymphoma characterised by the proliferation of large B-cell immunoblast-like neoplastic cells that demonstrate plasma cell differentiation associated with loss of conventional B-cell antigens [2,3]. Delecluse et al [4] originally described PBL in HIV-positive patients as a subtype of diffuse large B-cell lymphoma confined to the oral cavity. The neoplastic cells display frequent association with Epstein-Barr virus (EBV) infection. EBV-encoded RNA (EBER) in-situ hybridisation positivity has been described in 60-75% of PBLs [2,5]. HIV-positive patients frequently have more EBV-positive PBLs compared with HIV-negative patients [5-6].

PBLs have also been reported in the anorectal region, gastrointestinal tract, skin, lung, nasopharynx, paranasal sinuses, lymph nodes, breast, central nervous system and soft tissues [7-8].
Several studies have also reported on PBLs in HIV-negative patients with and without immunosuppression [9-12]. PBLs arising in HIV-negative and HIV-positive patients have different clinicopathological characteristics which include younger age, male predominance and better response to chemotherapy in HIV-associated PBLs [9].

PBLs can be subdivided into two morphologic subgroups including lymphomas comprised of a monomorphic population of immunoblasts and lymphomas with plasmacytic differentiation composed of immunoblasts, plasmablasts and cells showing plasma cell differentiation [2,8]. Extra-oral PBLs are associated with non HIV-related immunosuppression and commonly display plasmacytic differentiation [8].

Cytogenetic studies have found c-MYC translocation in 50% of PBLs cases and this is associated with MYC-protein overexpression [2,13]. EBV-positive neoplasms are more likely to have c-MYC rearrangements when compared to EBV-negative neoplasms [13].

A considerable body of literature on oral plasmablastic lymphoma is available [4,14-17]. However, the histopathological features and descriptions of extra-oral PBLs are infrequently reported particularly in the stomach, small intestine and colon. The reports currently available include isolated cases and small series of up to 4 cases [18-26]. As a result, there is a need for a comprehensive examination of PBLs in these segments of the gastrointestinal tract to broaden the reported cases and improve on diagnostic accuracy and promote early intervention.
2. AIM AND OBJECTIVES

2.1. Aim of Study

The aim of this study was to assess the histopathological spectrum of gastrointestinal tract plasmablastic lymphomas in HIV-infected adult patients.

2.2. Specific Objectives

The objectives were to assess:

i. the histomorphological heterogeneity, if any;

ii. the immunophenotypic profile;

iii. the EBV status;

iv. the cytogenetic alterations.
3. LITERATURE REVIEW

3.1. Definition of Plasmablastic Lymphoma

Plasmablastic lymphoma is an aggressive, high-grade B-cell non-Hodgkin lymphoma characterised by diffuse proliferation of large neoplastic cells which resemble B-cell immunoblasts [2]. The neoplastic cells display terminal B-cell or plasma cell differentiation associated with acquisition of a variety of plasma cell antigens and loss of conventional B-cell antigens [2,27-28].

3.2. Classification of Plasmablastic Lymphoma

The WHO Classification of Haematopoietic and Lymphoid Tumours classifies neoplasms mainly according to their cell of origin, after which distinct disease entities within each category are further defined according to their histomorphology, immunophenotypic profile and cytogenetic alterations.

The World Health Organisation (WHO) classifies plasmablastic lymphoma as a separate entity, distinct from DLBCL under mature B-cell neoplasms [2] (Table 3.1).
Table 3.1: WHO classification of mature B-cell neoplasms. (Adapted from the 2017 WHO classification of haematopoietic and lymphoid tumours)

3.3. History

Delecluse et al [4] originally described plasmablastic lymphoma in HIV-positive patients as a subtype of diffuse large B-cell lymphoma confined to the oral cavity. The neoplastic cells showed unusual immunophenotype (weak or absent CD45 and CD20), and frequent EBV positivity. The tumour cells expressed characteristic plasma cell markers (MUM-1, VS38c, CD79a). The B-cell nature of the neoplastic cells was confirmed by cytoplasmic expression of immunoglobulins (Ig) and monoclonal rearrangements of the Ig heavy chain genes. In
2008, this lymphoma was reclassified as a separate diagnostic entity, distinct from diffuse large B-cell lymphoma (DLBCL), not otherwise specified [2].

Subsequently, plasmablastic lymphomas have been reported at extra-oral sites such as anorectal region, gastrointestinal tract, skin, lung, nasopharynx, paranasal sinuses, lymph nodes, breast, mediastinum, central nervous system and soft tissues [7,29 -32]. The oral cavity however still remains the preferred site of involvement by PBL.

Several studies have reported on PBLs in immunocompetent and immunosuppressed HIV-negative patients [9,33]. PBLs arising in HIV-positive and HIV-negative patients have been shown to have different clinicopathological characteristics. HIV-positive patients have been shown to have better response to chemotherapy and better overall prognosis [9]. Similarities and differences have also been identified in clinicopathological characteristics of oral and extra-oral plasmablastic lymphomas (EPBL). Oral cavity PBLs have been found to be associated with HIV infection and plasmablastic morphology with no plasmacytic differentiation. Extra-oral plasmablastic lymphoma has been found to be associated with non-HIV related immunosuppression and commonly display plasmacytic differentiation [10].
3.4. Aetiopathogenesis

The aetiopathogenesis of this tumour has not yet been fully elucidated, but the plasmablast is known to be the cell of origin [3]. The current thinking is that the plasmablast is derived from a post-germinal centre B-cell that has undergone preterminal differentiation via somatic hypermutation and class switching recombination to resemble a plasma cell rather than a B-cell [3, 16, 28].

Cytogenetic alterations which have been reported include rearrangements involving c-MYC and the immunoglobulin gene and strong association with EBV infection [13]

Most studies have failed to show any association between plasmablastic lymphoma and Kaposi sarcoma-associated herpesvirus (KSHV) or human herpesvirus 8 (HHV8) [27].

3.5. HIV Infection

Human immunodeficiency virus (HIV) infection has been linked to increased risk of developing haematolymphoid disorders. Primary central nervous system lymphoma (PCNSL), primary effusion lymphoma (PEL), and plasmablastic lymphoma are some of the histopathological subtypes commonly encountered in HIV-infected patients [1].

Castillo et al [9] in a large review of 228 patients with plasmablastic lymphoma found 69% of the patients to be HIV-positive and 31% to be HIV-negative. Amongst the HIV-negative patients, approximately a third of the patients had some form of immunosuppression such
as an organ transplant or elderly with presumptive immunosenescence. The remainder of
the patients with plasmablastic lymphoma were HIV-negative and immunocompetent
[9,33].

In HIV-positive patients, several pathogenetic mechanisms such as chronic antigen
stimulation, cytokine deregulation (Interleukin 6) and the role of EBV and HHV8 have been
proposed [1]. HIV-infected macrophages up-regulate the expression of Interleukin 6 (IL-6)
which is an important plasma cell growth factor [34-35].

EBV and/or HHV-8 infection, which are common in HIV-infected patients, are also
associated with high serum levels of IL-6 which may further explain the common association
of plasma cell neoplasms in HIV-patients [34-35].

3.6. Clinical Features

HIV-associated oral PBL is recorded in literature as occurring more frequently in males and
at a mean age of 39 years [36-38]. Conflicting reports on the mean age and sex preference
have reported in the published case reports and small case series of gastrointestinal PBL.
Luria et al [25] in a review of published cases of gastrointestinal PBL, reported a mean
patient age of 57 years (range: 17 - 82 years) with a male-to-female ratio of 3:4.
Komaranchath et al [23] reported on four cases which showed a mean age of 29.5 years
(range: 13 - 45 years) with a male-to-female ratio of 3:1.
The oral lesions on the palatal mucosa and gingiva typically present as rapidly growing fleshy masses [4, 37,38]. In extra-oral locations, plasmablastic lymphoma present, most commonly as mucocutaneous masses, which may ulcerate or bleed [2,9]. Generalised lymphadenopathy is an unusual clinical presentation [2,9]. In the gastrointestinal tract, the common presentations include non-specific abdominal symptoms associated with polypoid masses or ulcerating masses [22-24].

According to Lurai et al [25], locations of the primary lesions within the gastrointestinal tract consist of the stomach (43%), small intestine (21%), anal region (21%), caecum (14%), colon (7%), and oesophagus (7%) [25].

**3.7. Morphology**

The characteristic immunoblasts exhibit abundant basophilic cytoplasm with occasional paranuclear hofs, round and oval nuclei with vesicular chromatin and prominent central nucleoli, while the plasmablasts show eccentrically placed nuclei with coarse chromatin and small conspicuous nucleoli [4,8,38-40].

Tumours showing plasmacytic differentiation show a predominant population of plasmablasts/immunoblasts with a small component of small plasma cells containing a clock-face chromatin pattern, amphophilic cytoplasm and a paranuclear hof [8].
Figure 3.1: Illustration of a plasmablast

PBLs can be subdivided into two morphological subgroups based on cellular features. The first group ‘PBL of the oral mucosa type’ is composed of a monomorphic population of plasmablasts/immunoblast with no/minimal plasmacytic differentiation (2,8). The other group, ‘PBL with plasmacytoid differentiation’ shows plasmacytic differentiation and is composed of plasmablasts and cells showing plasma cell differentiation (2,8).

The presence of scattered tingible body macrophages result in a “starry-sky” appearance [2]. Background small mature lymphocytes, brisk mitotic activity, apoptotic bodies and necrosis are common features in PBL of the oral cavity [2,4].
3.8. Immunophenotype

The neoplastic cells of PBLs show immunophenotypic profile compatible with late or terminally differentiated B-cell type. The tumour cells show weak or no expression of leukocyte common antigen (CD45) and loss of mature B-cell markers (CD20, CD19 and PAX5) [4,8]. However, the plasma cell markers (VS38c, CD38, MUM1, EMA and CD138) are expressed [2,41]. CD79a and CD30 are also frequently positive and Cyclin D1 is negative. CD56 is detected in 25% of cases [2].

Cytoplasmic immunoglobulins (usually IgG) and immunoglobulin light chain restriction (kappa or lambda) suggestive of clonality have been described in PBLs [2].

The phenomenon of T-cell infidelity in which PBLs, which are B-cell lymphomas, express T-cell antigens (CD3, CD4, CD43, and CD7) is well described [18,42,43]. The proposed possible mechanisms of this lineage ambiguity include trans-differentiation and downregulation of B-cell transcription factors (particularly PAX5) [44]. CD3 expression in CD20+ B-cell lymphomas is usually not problematic since co-expression of these two markers will prompt further workup with more lineage-specific markers and molecular studies. However in cases when common B-cell antigens (CD20 and PAX5) are negative, T-cell infidelity may result in the tumour being classified as a peripheral T-cell lymphoma, not otherwise specified (PTCL NOS).
Plasmablastic lymphomas are a high grade neoplasm with associated high proliferation indices (Ki-67/MIB1) of >90% [2].

3.1. EBV Status

The exact relationship between EBV status and PBL is still evolving. In EBV-infected B-cells, the linear viral genome becomes circular and persists within the cells as an episome. The virus can express genes in two distinct programmes, the latent or lytic cycle [45].

A limited set of genes are expressed during the latent cycle and these include EBV nuclear antigens (EBNAs) 1, 2, 3A, 3B, 3C and leader protein (LP) and three latent membrane proteins (LMPs), which are associated with transforming activity [45].

EBV-associated neoplastic diseases can be divided into three patterns of latency depending on the viral genes expressed [27]. In Type I (e.g. Burkitt lymphoma), only EBNA-1, non-translated EBV-encoded RNAs (EBERs) and BamH1 A rightward transcripts (BARTs) are expressed. Type II (e.g. Hodgkin lymphoma and primary effusion lymphoma) expresses EBNA-1, LMP1, LMP2, EBERs and BARTs. In type III (e.g. post-transplant lymphoproliferative disorders, PTLD), all latency genes are expressed.

LMP1 is the major transforming protein of EBV and upregulates expression of the anti-apoptotic proteins Bcl-2 and A20, and stimulates cytokine production (IL-6 and IL-8) [45].
EBNA-1 is a nuclear phosphoprotein that allows the EBV genome to be maintained in the B-cell by binding to viral DNA [46]. EBNA-2 is a viral transcription factor that enhances the growth and transformation of B-cells by inducing expression of LMP1 and LMP2 [47]. EBNA-3s upregulate expression of cellular proteins [38]. EBERs do not encode for any protein but are thought to be important in oncogenesis and resistance to apoptosis [47].

Delecluse et al [4] originally described the association between plasmablastic lymphoma and latent EBV infection. The World Health Organisation (WHO) Classification cites EBER ISH as positive in 60 - 75% of cases [2]. A higher association (86%) between PBL and EBV was also reported by Castillo et al [9] who showed that 82% of the HIV-positive cases and 46% of the HIV-negative cases, displayed expression of EBER by ISH. EBV infection is higher in PBL classified as ‘PBL of the oral mucosa type’ as compared to incidence in ‘PBL with plasmacytic differentiation [2,34]. Majority of the PBL cases have shown a type-1 EBV latency pattern [49,50]. EBV has also been detected in 15% of cases of extramedullary plasma cell tumours of the head and neck in immunocompetent patients [8].

3.2. Cytogenetic Studies

Conventional cytogenetic studies typically reveal clonal rearrangement of IGH and polyclonal T-cell receptor (TCR). The other molecular alterations which have been identified are c-MYC-translocation and MYC-amplification [13]. Translocation involving MYC gene has been reported in 60% of plasmablastic lymphoma cases and this results in MYC-protein overexpression [13]. The partner for MYC is IGH in most cases (>90%) [5,28,51-53]. MYC
rearrangements are more commonly found in the EBV-positive cases (74%) than in the EBV-negative cases (43%) [13]. The MYC rearrangements have also been found to be more common in HIV-positive patients [53].

A recent study has demonstrated recurrent somatic mutations in PRDM1 in 50% of plasmablastic lymphoma cases (8 of 16 cases evaluated) [41]. PRDM1 normally helps in regulation of MYC and is also a driver of terminal B-cell differentiation. Mutation of PRDM1 in the setting of PBL may contribute to dysregulation of MYC, while apparently retaining the capacity to induce terminal B-cell differentiation [41].

Cytogenetic studies have not detected rearrangements of oncogenes altered in other lymphomas (BCL2, BCL6, MALT1, and PAX5) [13].

3.3. Differential Diagnosis

Diagnostic challenges for PBL may arise in the gastrointestinal tract due to its morphology, lack of expression of CD45 and pan-B cell markers and occasional T-cell infidelity. This immunophenotype can inadvertently lead to the exclusion of lymphoma from the differential diagnoses. Misdiagnosis of gastrointestinal tract PBL would have a great impact on treatment strategy and clinical outcome.

Morphologically, the neoplasm may mimic a diffuse large B-cell lymphoma (DLBL), anaplastic lymphoma kinase (ALK) positive large B-cell lymphoma, extracavitary primary
effusion lymphoma (EPEL), plasmablastic myeloma, poorly differentiated carcinoma (including colorectal carcinoma) and melanoma [54].

Diffuse large B-cell lymphoma not otherwise specified (DLBCL NOS) may have similar morphological features to PBL but is distinguished from PBL through its expression of pan-B-cell markers (CD20, CD19, CD79a, PAX5) [55-57]. The tumour cells in DLBCL NOS may not express one or more of these pan B-cell markers [57].

ALK-positive large B cell lymphoma is a rare aggressive neoplasm composed of ALK-positive, large monomorphisc immunoblast-like B-cells [58-59]. The cells usually lack pan B-cell markers (CD20, CD19, CD79a and PAX5) and express plasma cell phenotype (MUM-1, VS38c, CD138). All the cases are also EBV and HHV8 negative [58-59].

Extracavitary primary effusion lymphoma (PEL) and PBL have overlapping histopathological features and immunophenotypic profile, however, the presence of HHV8 infection supports the diagnosis of extracavitary PEL [60-62]. Extracavitary PEL displays a higher expression of CD20 and CD79a when compared to PBLs [60-62].

Differentiating PBL from plasmablastic myeloma remains a major diagnostic challenge and is at times impossible. The two entities may have similar morphology, immunophenotype and more importantly clinical features [63]. Some have proposed that PBL may actually represent an aggressive form of multiple myeloma or plasmacytoma [63]. The presence of a
high proliferative index (Ki-67) and EBV infection favours PBL whilst immunopositivity for Cyclin D1, CD117 and CD56 favours a plasmablastic myeloma [2, 63,64]. The absence of serum monoclonal proteins, osteolytic bone lesions and/or bone marrow involvement, the presence of EBV infection, HIV/AIDS-related immunodeficiency, and the aggressive clinical course favours the diagnosis of PBL above other plasma cell neoplasms [63]. However multiple myeloma has also been described in HIV/AIDS patients with or without EBV infection [53,63,65].

The combination of CD45, pan T-cell and pan B-cell marker immunonegativity, associated with EMA immunopositivity may lead to a diagnostic pitfall of an undifferentiated carcinoma. Carcinomas will however show positivity for broad-spectrum cytokeratin and not express plasma cell markers (MUM1, Vs38c, CD138) or show kappa light chain restriction. Aberrant cytokeratin expression has been reported in a small subset of PBLs [33,67]. Aberrant CD138 staining has also be described in undifferentiated carcinomas [67].

Based on immunohistochemical profile a, melanoma can be excluded as it stains with S100, Melan A, HMB45, MiTF and SOX10, all of which are negative in PBLs.

### 3.4. Treatment and Prognosis

The tumour is highly aggressive with poor response to therapy and short survival despite aggressive therapy combining highly active anti-retroviral (HAART), systemic chemotherapy and/or radiotherapy.
Cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) and CHOP-like regimens are considered inadequate due to highly aggressive nature of the neoplasm, more intensive regimens, such as infusional EPOCH (etoposide, vincristine, doxorubicin, cyclophosphamide, and prednisone) have been recommended [36]. Due to the lack of CD20 expression by plasmablastic cells, the use of rituximab, an anti-CD20 monoclonal antibody, is not currently a therapeutic standard [8].

Patients with plasmablastic lymphoma and HIV infection have been found to have an overall survival of 14 months compared to 9 months in HIV-negative patients [9].

3.5. Current Status

Few cases of gastrointestinal tract plasmablastic lymphoma have been reported globally. The diagnosis is commonly based on morphological features and immunohistochemical profile seen at the commonly described sites.

The gastrointestinal tract is a unique location with its own specific differential diagnoses which have to be considered in this location. This study therefore seeks to assess the histomorphological spectrum of plasmablastic lymphoma in the gastrointestinal tract and to highlight the microscopic pitfalls and mimicry, if any.
4. MATERIALS AND METHODS

4.1. Study Population

The study design was that of a laboratory-based retrospective study which involved purposive sampling and evaluation of all biopsies diagnosed and coded as ‘plasmablastic lymphoma’ from 1st January 2009 to 31st December 2018. The cases were accessed from the archive of the Department of Anatomical Pathology, Inkosi Albert Luthuli Central Hospital (IALCH), National Health Laboratory Service (NHLS), and School of Laboratory Medicine and Clinical Sciences, Durban, KwaZulu-Natal, South Africa, using the SNOMED word and code search engines.

4.2. Study Sample and Sampling Strategy

This retrospective study included all cases diagnosed as PBL from the gastrointestinal tract (oesophagus, stomach, small intestine and colon) from 1st January 2009 to 31st December 2018. A total of 18 cases were retrieved from the departmental archives.

On histopathological and immunohistochemical profile review, one case was re-classified as diffuse large B-cell lymphoma (DLBCL). This case was excluded from the study. The study sample then comprised 17 cases of gastrointestinal tract PBL.
Demographic data extracted from the NHLS laboratory information system (Trakcare) and histopathology reports of the patients included their age, gender, HIV status and biopsy site (stomach, small intestine and colon). Tumour size was also retrieved from the histopathology reports.

4.3. Inclusion / Exclusion Criteria

The inclusion criteria were those cases from HIV-positive patients that had a histopathological and immunophenotypic profile compatible with a diagnosis of PBL and that appeared to have occurred along the gastrointestinal tract site as ascertained from the demographic data available on the histopathology reports and microscopic features. Patients with plasmablastic lymphoma from the oral cavity and anorectal region were excluded from the study.

4.4. Data Collection Methods and Tools

Stored haematoxylin and eosin (H&E) stained sections, immunohistochemical sections and cytogenetics study images were reappraised. Patient slides were allocated study numbers in order to maintain patient confidentiality. Two pathologists (A.M, G.N) independently reappraised stored sections on Olympus BX43 (Olympus, Tokyo, Japan) microscopes. Whenever a discrepancy occurred, both investigators re-examined the slides to reach a consensus. Data sheets were used for data collection.

The diagnostic criteria that were used for the confirmation of PBL included a high-grade neoplasm consisting of cells demonstrating variable immunoblastic, plasmablastic and/or plasmacytic features. In addition, the neoplastic cells were expected to demonstrate CD20
immunonegativity and at least one plasma cell marker (MUM-1, VS38c, CD138) immunopositivity. The characteristic immunoblast exhibit round to oval nuclei with vesicular chromatin and prominent central nucleoli while the plasmablast show eccentrically placed nuclei with coarse chromatin and small conspicuous nucleoli [8]. Tumours showing plasmacytic differentiation show a predominant population of plasmablasts/immunoblast with a small component of small plasma cells containing a clock-face chromatin pattern, amphophilic cytoplasm and a paranuclear hof [8].

4.4.1. Histomorphology

The original 4μm-thick haematoxylin and eosin (H&E) stained slides were reviewed to evaluate the following histopathologic features: architectural growth patterns (diffuse, nodular, trabecular and/or single cells) and cytomorphology (plasmablast, immunoblast, centroblast and presence of plasmacytic differentiation). The mitotic activity (expressed as mitotic figures per 10 high power fields in the most mitotic area, using a 40 X objective and a 10 X ocular lens; field area: 0.237 mm2), apoptotic bodies and tumour necrosis were also evaluated.

Where necessary, re-cuts at 4μm were done from formalin-fixed paraffin embedded (FFPE) archived tissue blocks. Re-staining of archived slides was also done in some cases where the paraffin-embedded archived blocks could not be retrieved or when there was no residual tissue on the block.
4.4.2. Immunohistochemistry

Immunohistochemical slides used to establish the initial diagnosis by the primary pathologist were reviewed. Immunohistochemical stains commonly assessed on the biopsies include LCA (CD45), CD3, CD20, MUM1/IRF4, CD138, VS38c, CD79a, PAX5, CD30, HHV8, ALK-1, AE1/AE3, CAM 5.2 and MNF116.

Where necessary and sufficient tissue available, stains were repeated using manufacturer’s instructions on an automated immunostainer (Ventana Benchmark Ultra, Tucson, AZ). The repeat immunohistochemistry monoclonal antibodies were performed on formalin-fixed paraffin embedded 3-μm sections (Table 4.1). Appropriate positive controls were used throughout.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Antigen Retrieval</th>
<th>Control</th>
<th>Supplier</th>
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</thead>
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<td>Roche</td>
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<td>Roche</td>
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<td>High pH</td>
<td>Lymph node</td>
<td>Roche</td>
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<td>High pH</td>
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<td>Cell Marque</td>
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<td>PC</td>
<td>RTU</td>
<td>High pH</td>
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<td>Dako</td>
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<td>High pH</td>
<td>Tonsil</td>
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</tr>
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<td>SP34</td>
<td>RTU</td>
<td>High pH</td>
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<td>Roche</td>
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<td>Dako</td>
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<td>13B10</td>
<td>RTU or 1:200</td>
<td>High pH</td>
<td>Skin with Kaposi sarcoma</td>
<td>Cell Marque</td>
</tr>
<tr>
<td>ALK-1</td>
<td>ALK01</td>
<td>RTU</td>
<td>High pH</td>
<td>Anaplastic lymphoma</td>
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<tr>
<td>CAM 5.2</td>
<td>1:10</td>
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<td>BD Biosciences</td>
<td></td>
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<tr>
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<td>30-9</td>
<td>RTU</td>
<td>High pH</td>
<td>Lymph node</td>
<td>Roche</td>
</tr>
</tbody>
</table>

RTU: Ready to use

Table 4.1 List of antibodies used for immunophenotypic analysis
Supplier Information

Dako - Glostrup, Denmark
BD Biosciences - San Jose, CA, USA
Roche - Basel, Switzerland
Abcam - Cambridge, United Kingdom
Cell Marque - Rocklin, CA, USA

Brown granular staining either on cell nucleus (MUM1/IRF4, Ki-67/MIB1, PAX5), cell membrane (CD138, CD45, CD20, CD3) or in cell cytoplasm (VS38c, ALK-1, CD79a, AE1/AE3, CAM 5.2, MNF116) was regarded as positive.

Cases were considered as positive for MUM1/IRF4 if 30% or more of the tumour cells were positively stained by the antibody, as described in the study by Hans et al [68]. The results for the rest of the markers were recorded and positive or negative.

Qualitative scoring of the intensity of staining was categorised as follows: “negative” (−), “weak” (+), “moderate”(++, and “strong”(+++) [69].

The proliferation index was assessed using Ki-67. Ki-67 was assessed by counting positive and negative cells in 5 high power fields, averaging and then converting to a percentage.

The archived IHC stains were utilised despite the fact that they lacked standardisation in-terms of antibody clonality, dilution and antigen retrieval methods. This was due to the financial implications which would have resulted from repeating the stains.
Some of the stains had been run manually using in-house modification of internationally recognised protocols with appropriate positive and negative controls.

**4.4.3. Epstein-Barr Virus in-situ Hybridisation Studies**

EBER staining assessment was done using stored slides which formed part of the diagnostic case record.

If necessary and where sufficient material was available, staining was repeated.

Chromogen *in situ* hybridisation was performed on 3-μm-thick sections for Epstein-Barr virus–encoded RNA (EBER) (INFORM EBER Probe, Ventana Medical Systems, Tucson, AZ, USA). Slides were stained on an automated immunostainer (Ventana Benchmark Ultra, Tucson, AZ) and for visualisation the ISH iView Blue Detection Kit (Ventana Medical Systems) with alkaline phosphatase was used. Nuclear Fast Red (Ventana Medical Systems) was employed for contrast.

Appropriate positive controls were used throughout. A positive signal appeared as an intense blue density of the cell nucleus. The result was recorded as either EBER positive or negative.

**4.4.4. Cytogenetic Studies**

Digital images for cytogenetic studies results were reviewed for two cases on which they had been conducted successfully. Fluorescence *in-situ* hybridisation (FISH) was performed with Vysis LSI MYC dual-colour, break-apart rearrangement probe (Abbott Molecular, Des
Plaines, IL, USA) and was carried out on 4μm-thick formalin-fixed, paraffin-embedded tissue sections following manufacturer’s guidelines. DAPI (4′, 6-diamidino-2-phenylindole) was used as a nuclear counterstain.

Results were evaluated on an Olympus BX61 (Olympus, Tokyo, Japan) immunofluorescence microscope using appropriate filters. Images were captured with Cytovision 4.0 (Leica Biosystems Inc., Buffalo Grove, IL, USA).

A normal nucleus hybridised with the LSI MYC Dual Colour Break Apart Rearrangement Probe displays a pattern of two orange/green fusion signals. In a cell with a translocation breakpoint, one green, one orange and one fusion signal pattern should be observed. The cut-off value for a positive result with the LSI MYC probe in the study was 10%.

Immunoglobulin gene rearrangements were investigated by polymerase chain reaction (PCR) on formalin-fixed, paraffin-embedded tissue according to the departmental standard operating procedures at that time, for one of the cases.

4.5. Study Location

This study was conducted in the laboratory of the Department of Anatomical pathology of the National Health Laboratory Service (NHLS) and University of KwaZulu-Natal, Inkosi Albert Luthuli Central Hospital (IALCH), Durban, South Africa.
4.6. Ethical Considerations

The Biomedical Research Ethics Committee of the University of KwaZulu-Natal approved the study (BREC Ref No: BE016/19). Furthermore, permission was also granted by the Department of Health (DOH), Province of KwaZulu-Natal and National Health Laboratory Service (NHLS) to retrieve and use archived material and data.

4.1. Data Analysis Techniques

The study design was that of a retrospective study that was based on descriptive demographics, histomorphological, immunohistochemical and *in-situ* hybridisation (ISH) data. Computerised statistical analysis and interpretation was performed using Stata 15.0 statistical software (Stata Corp LP, College Station, TX, USA). The data was descriptive and variably continuous and categorical, consisting of frequencies that were expressed in percentages and ratios to summarise data. Descriptive statistics included calculations of means. Differences in means between groups were evaluated using two sample, two-tailed t-tests. In all instances, a p-value of less than 0.05 was considered statistically significant.
5. RESULTS

5.1. Study Sample

A total of eighteen (18) cases diagnosed as gastrointestinal tract PBL were retrieved from the archives of the Department of Anatomical Pathology, National Health Laboratory Service, University of KwaZulu-Natal, Durban over a ten-year period between January 2009 to December 2018. One case was reclassified as a diffuse large B-cell lymphoma (DLBL) and excluded from the study, leaving seventeen (17) cases that made up the study cohort.

5.2. Patient Demographics

5.2.1. Sex

Thirteen (76%) patients were males (M) and only four (24%) were females (F). There was a male bias with a male-to-female of 3:1 (Figure 5.1).
5.2.2. Age

Age was documented for 16 of the 17 patients recruited into the study cohort.

Gastrointestinal tract PBL documented over the study period occurred between the ages of 29 and 68 years (mean 41 years, 95% CI = 35.35 - 46.65) (Figure 5.2). The mean age of females in the study population was 30 years (95% CI = 26.82 - 38.7) and the mean age of males was 44 years (95% CI = 38.53 – 50.80). The difference in mean age between males and females was statistically significant (two sample t-test, p = 0.003).
Figure 5.2: Age distribution in males and females with gastrointestinal tract PBL

5.3. Nature of Biopsy

The biopsies were either endoscopic incisional biopsies (53%, 9/17) or excisional biopsies (47%, 8/17).
5.4. Anatomical Location

The gastrointestinal tract locations of the tumours, upon which the primary diagnosis was made at presentation, are listed in Table 5.1. Information regarding the tumour site was indicated in the macroscopic descriptions in the histopathology reports.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Years)</th>
<th>Race</th>
<th>Sex</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>Female</td>
<td>Small Intestine</td>
</tr>
<tr>
<td>2</td>
<td>42</td>
<td>Black</td>
<td>Male</td>
<td>Colon</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Black</td>
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<td>Small Intestine</td>
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<td>4</td>
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<td>Stomach</td>
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<tr>
<td>5</td>
<td>41</td>
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<td>Male</td>
<td>Stomach</td>
</tr>
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<td>53</td>
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<td>7</td>
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<td>41</td>
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<td>Colon</td>
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<td>49</td>
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<td>Small Intestine</td>
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<td>Male</td>
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<td>17</td>
<td>29</td>
<td>Black</td>
<td>Female</td>
<td>Stomach</td>
</tr>
</tbody>
</table>

Table 5.1: Summary of the clinical features of PBL cases included in the study
Gastrointestinal tract PBL occurred most commonly in the small intestines (42%, 7/17) then stomach (29%, 5/17) and the colon (29%, 5/17).

5.5. Gross Pathology

Minimal details on the gross appearance of the tumours were retrieved from the histopathology reports. Details on gross tumour size were available for six of the eight excisional biopsies. The tumours ranged in size from 3 to 8 cm (mean 5.5 cm; 95% CI = 3.54 - 7.46).

5.6. Histopathological Features

5.6.1. Architectural patterns

On microscopic appraisal, the neoplasm showed one or more architectural patterns with diffuse architecture being the most common (82%, 14/17), followed by scattered single cells (29%, 5/17), multinodular (24%, 4/17), trabecular (18%, 3/17) and pseudo-alveolar (12%, 2/17) growth patterns (Fig. 5.3 - 5.8). A low power “starry-sky” pattern was seen in the majority of excision biopsy specimens (75%, 6/8) (Figure 5.3). The incision biopsy specimens showed starry-sky appearance in 56% (5/9) of the cases.
Figure 5.3: PBL with a starry-sky appearance imparted by interspersed tingible body macrophages (arrows) (H&E, X100 magnification)

Figure 5.4: Small biopsy showing PBL with diffuse architecture (H&E, X100 magnification)
Figure 5.5: PBL with a multinodular architecture (H&E, X100 magnification)

Figure 5.6: PBL with a trabeculae architecture (H&E, X100 magnification)
Figure 5.7: PBL with tumour cells infiltrating lamina propria as single cells (arrows) (H&E, X200 magnification)

Figure 5.8: PBL with a pseudo-alveolar growth pattern (H&E X100 magnification)
5.1.1. Cytomorphology

All cases showed proliferation of large lymphoid cells with immunoblastic and plasmablastic morphology with occasional presence of paranuclear hof (Figures 5.9 - 11). Plasmacytic differentiation was identified in 29% (5/17) of the cases (Figure 5.12). Scattered centroblasts were identified in 11 (65%) cases. One case showed centroblasts as the predominant cell type (Figure 5.13). The majority of cases (71%) showed amphophilic cytoplasm while the remaining 29% exhibited eosinophilic cytoplasm. Two cases showed focal clear cell change (Figure 5.14). Scattered multinucleated tumour giant cells were observed in two cases (Figure 5.15).

Figure 5.9: PBL showing a plasmablastic morphology (H&E, magnification X400)
Figure 5.10: PBL showing a plasmablastic morphology (H&E, magnification X400)

Figure 5.11: PBL showing a plasmablastic and immunoblastic morphology (H&E, magnification X400)
Figure 5.12: PBL with plasmablastic phenotype and plasmacytic differentiation (arrows) (H&E, magnification X400)

Figure 5.13: PBL showing neoplastic cells with multiple nucleoli in keeping with centroblastic morphology (H&E, magnification X400)
Figure 5.14: PBL showing neoplastic cells with focal cytoplasmic clearing (H&E, magnification X400)

Figure 5.15: PBL showing scattered multinucleated giant cells (arrow) (H&E, magnification X400)
All cases in the present study had identifiable mitotic figures, ranging from 16 to 62 (mean 32) per ten high power fields. Apoptotic bodies were observed in all biopsies and these were arranged as single cells or as confluent areas of apoptotic bodies. Necrosis was evident in 71% (12/17) of the biopsies.

5.1. Immunohistochemical findings

Summarised in Table 5.2 are the immunohistochemical results.

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<tr>
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Key: +/-: Weak positive, +: Positive, −Negative, ND: Not done CK: Cytokeratin (AE1/3, CAM5.2, MNF116)

Table 5.2: Immunohistochemical characteristics of the gastrointestinal tract PBL cases

CD45/Leucocyte common antigen (LCA): CD45 stained positively in 59% of the cases (10/17) and was negative in 41% (7/17). Staining was membranous and was invariably weak.
CD3: Staining was negative in 94% of the cases (16/17), being positive in only one case which showed T-cell infidelity (Figure 5.16). Background reactive T-cells were identified in all the cases.

CD20: All the cases in this study were negative for this pan B-cell marker (Figure 5.17).

PAX 5 displayed focal weak nuclear staining in one (20%, 1/5) case. The rest of the cases were negative (80%, 4/5) for PAX5.

MUM-1: MUM-1 antibody was positive in 88% (14/16) of the cases (Figure.5.18). The staining was nuclear in location, diffuse and moderate to strong in intensity.

VS38c: Eleven (11) of 14 (fourteen) cases (79%) which were tested with this antibody were positive (Figure.5.19). Staining was cytoplasmic in location, diffuse in distribution and strong in intensity.

CD138: All the eight cases (100%) tested with CD138 were positive (Figure.5.20). Staining was cytoplasmic in location, diffuse in distribution and strong in intensity.

Human Herpes Virus-8: All of the 8 cases tested with this antibody were negative (Figure 5.21). Confirmed cases of Kaposi sarcoma were used as the external positive control.

ALK-1: All 10 cases (100%) in which this antibody was tested were negative (Figure 5.22)

The Ki-67 proliferative index examined in thirteen cases was high (>90%) in all the cases (Figure 3.23). The positive signal was nuclear in location, of moderate to strong intensity.

Thirty-three percent (5/15) of the cases showed patchy immunopositivity for CD30. All cases which were tested for broad-spectrum cytokeratins (7/17) and CD56 (3/17) were negative.
Figure 5.16: PBL demonstrating negative staining of tumour cells (CD3, magnification X400)

Figure 5.17: PBL showing negative staining of the tumour cells (CD20, magnification X400)
Figure 5.18 PBL showing positive nuclear staining of tumour cells (MUM1, magnification X400)

Figure 5.19: PBL demonstrating positive cytoplasmic staining in tumour cells (VS38c, magnification X400)
Figure 5.20: PBL demonstrating positive membranous staining in tumour cells (CD138, magnification X400)

Figure 5.21: PBL demonstrating negative staining of the tumour cells (ALK-1, magnification X400)
Figure 5.22 PBL showing negative staining of the neoplastic cells (HHV8, magnification X400)

Figure 5.23: PBL showing positive nuclear expression in >90% of the tumour cells (Ki-67, magnification X400)
5.1. EBV Status

EBV status: *In-situ* hybridisation, for EBER was positive in 53% (9/17) of the cases under review (Figure 5.13). The positive signal was nuclear in location and had a deep blue colour.

Figure 5.24 PBL showing nuclear expression in tumour cells (EBER ISH, magnification X400)
5.2. Cytogenetic Studies

Fluorescence in-situ hybridisation (FISH) for c-MYC rearrangements was successfully performed on one case which showed c-MYC translocation. BCL2 and BCL6 were intact. The case which had c-MYC translocation also had EBER ISH positivity. One case showed immunoglobulin heavy (IGH) chain clonality. The other cases in the study did not have cytogenetic analysis done (11/17) or it was unsuccessfully attempted (4/17).
6. DISCUSSION

Plasmablastic lymphoma (PBL) is a highly aggressive CD20 negative B-cell lymphoma that is increasingly reported in HIV-positive patients [2,70]. Although originally described in the oral cavity of HIV positive patients, occurrence in other sites and HIV-negative patients has been documented [3,7,9]. The neoplastic cells display terminal B-cell or plasma cell differentiation associated with acquisition of a variety of plasma cell antigens and loss of conventional B-cell antigens [2,3,28].

The majority of the gastrointestinal tract PBLs have been described in the stomach, small intestine and colon as case reports and small case series [18-26]. Herein, we report on 17 patients with gastrointestinal tract PBLs focusing on the morphological and immunohistochemical features.

The age of the patients with gastrointestinal tract PBL in this series ranged between 29 to 68 years with a mean age at diagnosis of 41 years, which is similar to that reported in oral PBL [4-5,12,36-38]. Females were significantly younger than males (mean age 30 vs. 44 years). There were no paediatric gastrointestinal tract PBL cases reported during the study period.

The male predominance (male-to-female ratio of 3:1) is similar to what has been reported in oral PBL and in one small case series of gastrointestinal tract PBL [16,23,36].
All the patients in the study were HIV-positive (as per inclusion criteria). However, it is important to note that, none of the gastrointestinal tract PBL which were retrieved from the departmental archives were from HIV-negative patients. The strong relationship between HIV-infection and PBLs is well documented and PBL is recognised as one of the HIV-associated lymphomas [1,71]. The pathogenesis of HIV-related lymphomas has been attributed to chronic antigen stimulation, cytokine (IL-6) deregulation and the role of EBV and HHV8 co-infection [1]. Highly active antiretroviral therapy does not appear to have an impact on this increased risk of developing HIV-associated haematolymphoid neoplasms [72].

The PBL neoplasms in this case series occurred in the stomach (29%), small intestines (42%) and the colon (29%). A systematic review of publications on gastrointestinal tract PBLs reported between 1993 and 2013 found 14 cases of which the stomach (43%) was the predominant location followed by the anal region (21%) and small intestine (21%) [25].

The major histopathological features of PBLs include a predominantly diffuse growth pattern, “starry-sky” appearance on low power, necrosis, apoptotic cells, and neoplastic cells showing predominantly immunoblastic and plasmablastic cytomorphology. The characteristic immunoblastic cells exhibit round to oval nuclei with vesicular chromatin and prominent central nucleoli while the plasmablastic cells show eccentrically placed nuclei with coarse chromatin and small conspicuous nucleoli [8]. The findings of the present study showed comparable features to those reported in the case studies and series on gastrointestinal tract PBLs [18-26].
Plasmacytic differentiation with a predominant population of plasmablasts/immunoblasts with a small component of small plasma cells containing a clock-face chromatin pattern, amphophilic cytoplasm and a paranuclear hof was evident in 29% of the cases. Though other reports have suggested that plasmacytic differentiation occurs more preferentially in extra-oral sites [10], others have not supported such observations [8,73].

In the current study, one case of PBL was notable for a predominance of centroblasts with multiple peripheral nucleoli. Similar features were reported in a previous case on oral cavity PBL with predominant centroblastic morphology [74]. Hirosawa et al [74] reported on a case of oral cavity PBL that showed proliferation of large cells with centroblastic morphology. The observation of predominant centroblasts in a case of gastrointestinal tract PBL, to the best of our knowledge, has not been previously described.

In small endoscopic incisional biopsies, neoplastic cells may show trabecular and single cell growth patterns and less frequently show “starry-sky” appearance. The diagnosis of PBL in these circumstances becomes a challenge as the pathologist is constrained with limited amounts of tissue (for morphology and immunohistochemical workup) and marked biopsy artefacts commonly associated with small biopsies.
An unusual histopathological growth pattern identified in two of the present cases is the pseudo-alveolar growth pattern. Vaubell et al [75] in their report on paediatric PBL cases described pseudo-alveolar growth pattern in some of their cases. This pattern seems to have been observed in excision biopsies, which suggest the possibility that this could have been due to tissue fixation artefact. However it remains an important pattern to recognise in PBL in this location so as to avoid misdiagnosis.

Two cases in the current study were remarkable for the striking clear cell features of the tumour cells, a feature that, to our knowledge, has not been previously described in gastrointestinal PBL. There are only a few cases of clear cell lymphomas described in the literature, including diffuse large B-cell lymphoma and primary cutaneous anaplastic lymphoma [76-78]. The origin of the clearing of the cells in this case is not clear and may represent a tissue fixation artefact.

Multinucleated giant cells, similar to those of our cases, have been described in T-cell-rich large B-cell lymphoma, peripheral T-cell lymphoma, angioimmunoblastic T-cell lymphoma and Hodgkin’s disease [79]. Tumour giant cells have been reported before in a paediatric case series of PBL [75]. However, multinucleated giant cells identified in the current study in gastrointestinal PBL are a new finding.
Fifty-nine percent (59%) of the cases showed immunopositivity with CD45 (LCA) with quality of staining predominantly being weak and focal. This is in keeping with findings in other reports [2,73].

All cases in this study cohort showed negative staining with the CD20 antibody. One case showed focal weak nuclear staining with PAX5. A minority of cells may show weak staining with pan B-cell marker (CD20, PAX5) in some cases and this has been documented in literature [2].

Sixteen out of the seventeen cases (94%) showed negative CD3. Background scattered reactive T-lymphocytes were seen in all cases. One case showed aberrant T-cell antigen (CD3 and CD4) expression with negative staining for CD2, CD5 and CD8.

The phenomenon of T-cell infidelity/promiscuity in which PBLs, which are B-cell lymphomas, express T-cell antigens is well recognised phenomenon [18,42,43]. CD3 expression in CD20+ B-cell lymphomas is usually not problematic since co-expression of these two markers will prompt further workup with more lineage-specific markers and molecular studies. However in cases when common B-cell antigens (CD20 and PAX5) are negative, T-cell infidelity may result in the tumour being classified as a peripheral T-cell lymphoma, not otherwise specified (PTCL NOS).

The proposed possible mechanisms of this lineage ambiguity include trans-differentiation and downregulation of B-cell transcription factors (particularly PAX5) [44].
All the cases in the study had at least one plasma cell marker (MUM1, Vs38c, CD138) immunopositivity, indicating plasma cell differentiation. MUM1 was the most reliable plasma cell marker in this series with 87.5% (14/16) of the cases showing immunopositivity.

All of the cases in this series which were tested for HHV8 or ALK-1 were negative and this helped to distinguish the neoplasm from the other differential diagnoses.

The exact role of EBV in the pathogenesis of PBL is yet to be elucidated. EBER ISH expression in the current study, which was confined to PBLs in the gastrointestinal tract of HIV-positive patients, showed positivity in only 53% of the cases. This is at variance with rates seen in HIV-associated oral cavity and other extra-oral cavity PBLs (60-75%) [2,11,16]. Conflicting reports on the association between EBV-infection and gastrointestinal tract PBL have been reported in the published case reports and small case series of gastrointestinal PBL. Luria et al [25] reported on four cases in which all the three cases tested for EBER-ISH were positive. Komaranchath et al [23] reported on four cases in which LMP1 done to assess for EBV co-infection and only one case was positive. EBER was not done in this case series by Komaranchath et al [23].

The results in the current study further indicate that EBV infection is not the sole driver of this neoplasm. Although the sample size in the present study was small, the findings suggest that the EBV infection rates may be lower in the gastrointestinal tract PBLs when compared to other extra-oral and oral cavity PBLs.
Only two cases had documented molecular study results in the present study cohort. One case showed c-MYC rearrangement and the other case showed heavy chain clonality. Rearrangements of BCL-2 and BCL-6 were not detected. The case which had c-MYC translocation also had EBER ISH positivity. The findings are similar to what has been described in other studies [13]. MYC is an oncogene involved in the pathogenesis of aggressive haematolymphoid neoplasms and is usually activated by gene translocations [80]. MYC translocations are considered the primary genetic event in Burkitt lymphoma (BL) and also occur in diffuse large B-cell lymphoma (DLBCL) and high-grade B-cell lymphomas with MYC and BCL2 and/or BCL6 rearrangement (double or multi hit lymphomas) [1]. MYC rearrangements are associated with poor prognosis [80]. Although MYC rearrangement may play a critical role in the pathogenesis of gastrointestinal tract PBL in HIV-positive patients, further studies are required to understand the underlying mechanisms.

Accurate diagnosis of PBL and its distinction from other neoplasm which may have different management and better prognosis is important. Morphologically, the neoplasms which need to be distinguished from gastrointestinal PBL include diffuse large B-cell lymphoma (not otherwise specified), ALK + large B-cell lymphoma, extracavitary primary effusion lymphoma (PEL), plasmablastic myeloma, poorly differentiated carcinoma (including colorectal carcinoma) and melanoma [54].

Whereas most differentials can be distinguished from PBL based on morphology and immunohistochemical profile, plasmablastic myeloma provides a great diagnostic challenge and at times is impossible to distinguish from PBL. As a result some have suggested that PBL
may actually represent an aggressive form of multiple myeloma or plasmacytoma [61]. The presence of a high proliferative index (Ki-67) and EBV infection favours PBL whilst immunopositivity for Cyclin D1, CD117 and CD56 favours a plasmablastic myeloma [2,63,64]. In attempting to distinguish the two entities, correlation with clinical and radiological findings is critical [71].

6.1. Limitation of the Study

The study met several challenges during its operation which affected the quality of the study in certain aspects. This was a single-centre; laboratory based retrospective study with a relatively small number of cases. Due to lack of clinical correlation, the exact location of the primary disease could not be confirmed. The biopsy site documented on the histopathology report was taken as the primary site of the disease and the possibility of PBL occurring concurrently at other sites including oral cavity could not be excluded.

In some cases, tissue blocks could not be retrieved or had no tissue available for re-cuts. In such cases the haematoxylin and eosin (H/E) slides, had to be re-stained which compromised on the quality of slides and subsequent images.
Immunohistochemical stains on original sections at the time of diagnosis were done at two different laboratories, at different times over a ten-year period. Laboratory conditions at the time of diagnosis were different to the conditions at the time of the study. The archived IHC stains which were utilized lacked standardization in terms of antibody clonality, dilution and antigen retrieval methods. This could give rise to some variation in the results recorded. The option to use the original slides was due to the financial implications which would have resulted from repeating the stains to have a single fresh run.

Only two cases had molecular studies documented in the histopathology reports and laboratory records. The other cases in the study did not have cytogenetic analysis done (11/17) or it was unsuccessfully attempted (4/17). The molecular studies for the two cases were documented and images captured with Cytovision 4.0 (Leica Biosystems Inc, Buffalo Grove, IL, USA). However, the images could not be retrieved successfully as the computer was corrupted. The hard copies of the images were of poor quality and could not be copied. Four cases had molecular studies attempted unsuccessfully.
7. CONCLUSION AND RECOMMENDATION

This study represented the largest case series of gastrointestinal tract plasmablastic lymphomas published to date.

The study confirmed that gastrointestinal tract PBLs have similar sex distribution (male predominance), histomorphology and immunophenotype to that of oral and anorectal plasmablastic lymphoma. However, the stomach, small intestine and colon being uncommon locations, requires a high index of suspicion for an early accurate diagnosis and avoidance of potential diagnostic errors.

We have also described additional observations not previously described or emphasised in literature which includes pseudo-alveolar growth pattern, centroblast-predominance, multinucleated giant cells and clear cell change.

The study also provides evidence that EBV infection is not the sole driver in the pathogenesis of this neoplasm. The EBV infection showed less frequent association with the gastrointestinal tract PBL when compared to oral PBLs. Further research aimed at elucidating the true relationship between HIV/AIDS, EBV infection and PBL of the gastrointestinal tract in a detailed histopathology, molecular pathology and virology studies is recommended.
MYC rearrangement may play a pivotal role in the aetiopathogenesis of gastrointestinal tract PBL in HIV-positive patients, but further studies are required to understand the underlying mechanisms.

Differential diagnoses which show overlapping histopathological and immunological features with gastrointestinal tract PBL should always be considered. Clinicopathological correlation with clinical findings, imaging studies, histomorphological features, immunophenotype and EBV status are critical for establishment of an accurate diagnosis.
REFERENCES


APPENDIX 1: Ethics Clearance

UNIVERSITY OF KWAZULU-NATAL

13 March 2019

Dr A Mwazha
School of Laboratory Medicine and Medical Sciences
College of Health Sciences
amwazha@gmail.com

Dear Dr Mwazha

Protocol: Gastrointestinal tract plasmablastic lymphoma in HIV infected adults: A Histopathological Audit
Degree: MMed

EXPEDITED APPLICATION; APPROVAL LETTER

BREC Ref No: BE016/19

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 09 January 2019.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 07 March 2019 to BREC letter dated 15 February 2019 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given full ethics approval and may begin as from 13 March 2019. Please ensure that site permissions are obtained and forwarded to BREC for approval before commencing research at a site.

This approval is valid for one year from 13 March 2019. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.


BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee’s decision will be noted by a full Committee at its next meeting taking place on 09 April 2019.

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc: Supervisor: randaipj@gmail.com Postgrad Admin: dudhrzo@ukzn.ac.za

UNIVERSITY OF KWAZULU-NATAL

Biomedical Research Ethics Committee
Professor V Rambiritch (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X0400, Durban 4000
Telephone: +27 (0) 31 260 3466 Facsimile: +27 (0) 31 260 4609 Email: brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.asp

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67
Dear Dr A Mhwaza

Approval of research

1. The research proposal titled ‘Gastrointestinal tract plasmablastic lymphoma in HIV-infected adults: A histopathological audit’ was reviewed by the KwaZulu-Natal Department of Health.

The proposal is hereby approved for research to be undertaken at Inkosi Albert Luthuli Central Hospital NHLS Laboratory.

2. You are requested to take note of the following:
   a. Kindly liaise with the facility manager BEFORE your research begins in order to ensure that conditions in the facility are conducive to the conduct of your research. These include, but are not limited to, an assurance that the numbers of patients attending the facility are sufficient to support your sample size requirements, and that the space and physical infrastructure of the facility can accommodate the research team and any additional equipment required for the research.
   b. Please ensure that you provide your letter of ethics re-certification to this unit, when the current approval expires.
   c. Provide an interim progress report and final report (electronic and hard copies) when your research is complete to HEALTH RESEARCH AND KNOWLEDGE MANAGEMENT, 10-102, PRIVATE BAG X9051, PIETERMARITZBURG, 3200 and e-mail an electronic copy to hrkm@kznhealth.gov.za
   d. Please note that the Department of Health shall not be held liable for any injury that occurs as a result of this study.

For any additional information please contact Ms G Khumalo on 033-395 3189.

Yours Sincerely

[Signature]

Dr E Lutge
Chairperson, Health Research Committee
Date: 29/06/2020

Fighting Disease, Fighting Poverty, Giving Hope
8 March 2019

Dear Dr. A Mwazha,

RE: Access to NHLS informatics system within department for research – BE016/19

Title of study: Gastrointestinal tract plasmablastic lymphoma in HIV-infected adults: A histopathological audit.

As HoD representative for the NHLS KZN IALCH academic complex, I hereby support your request to use the data generated from specimens analysed in the IALCH Anatomical Pathology laboratory to extract laboratory test data that is relevant to the research. The data will be accessed by the investigator Dr Mwazha utilizing either the NHLS Central Data Warehouse and/or the NHLS laboratory information system (Trakcare). Strict confidentiality will apply with only the investigator and supervisors having access to research data.

Kind regards,

[Signature]

Dr. Verena Gounden, MBChB, FCPATH (Chem Path), MMed (Chem Path), DABCC
HoD representative NHLS KZN IALCH academic complex
Head of Department: Department of Chemical Pathology, IALCH
Tel: 031 240 2573 | Fax: 031 240 2576
verena.gounden@nhls.ac.za | www.nhls.ac.za

Chairperson: Prof Eric Sixhule Acting CEO: Dr Kamani Chetty
Physical Address: 1 Hoddartbreen Road, Sandton, Johannesburg, South Africa
Postal Address: Private Bag X3, Sandton, 2131, South Africa
Tel: +27 (0) 11 066 6000 / 0606 0014 (804657) www.nhls.ac.za
Patient number: 500006
## APPENDIX 4: Data Collecting Sheet

**DATA SHEET**

**STUDY NO.**

**Age:**

**Sex:**

**Race:**

### Primary Localisation

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### Other Features

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### Translocations

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## APPENDIX 5: Summary of Clinicopathological Features

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- **PCd**: Plasmacytic Differentiation
- **ND**: Not Done
- **US**: Unsuccessful
- **CK**: Cytokeratins