Dominant genetic aberrations and coexistent EBV infection in HIV-related Oral Plasmablastic lymphomas

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Key words

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SUMMARY

We present common cytogenetic features in the largest cohort of plasmablastic lymphoma (PBL) of the oral cavity published to date. This cohort included 45 patients, 32 of whom had a known HIV status, of which thirty one were HIV positive. Ninety eight per cent of all PBL cases were known to be EBV positive. In line with previous studies, we found that rearrangements of the *MYC* gene was the most common genetic abnormality seen in 60% of cases with the immunoglobulin heavy chain (*IGH*) locus as a partner in 51% of cases. Additional complex genetic aberrations were frequent, in particular, an increased copy number of the *CCND1* gene was seen in 41% of cases with true amplification of *CCND1* in 15% of cases. Aneuploidy was also observed for the *BCL6* gene in 28% of cases. Interestingly, rearrangements of both *IGH* genes were detected in 16% of cases with t(14;18) and t(11;14) respectively involved in conjunction with a t(8;14) in two cases. These bi-allelic *IGH* rearrangements have not been described before in oral PBL. Our results reinforce the notion that EBV infection and *MYC* rearrangements are important events in the pathogenesis of oral PBL. The genetic diversity and complexity observed in these cases, underlines the importance to genetically characterise PBL patients at presentation as this may inform the choice of more effective treatment modalities.

Introduction

Plasmablastic lymphoma (PBL) is a highly aggressive tumour that consist of large, neoplastic terminally differentiated B-cells¹. This lymphoma is currently recognised as a distinct entity with well documented morphological and immunophenotypic features². However, the pathogenesis of this disease has not been fully elucidated and the normal cell counterpart from which PBL takes origin is still to be determined. It is difficult to distinguish PBL from extramedullary plasmacytomas (EMPC) or multiple myeloma (MM) with plasmablastic morphology and the possibility that PBL may be related to these neoplasms has previously been proposed^{1,3,4}.

Most PBL cases are associated with immunodeficiency, and particularly HIV/AIDS related immunodeficiency. Infections with other viruses, especially the Epstein Barr virus (EBV) are frequently associated with PBL and the reported incidence vary from 60 to 100%^{1,3,5}, but the role of EBV in the pathogenesis remains unexplained. A possible aetiological role of the human herpesvirus-8 (HHV-8) has previously been proposed⁶⁻⁸ but the presence of HHV-8 currently rules against the diagnosis of PBL^{1,2}.

Recently Valera *et al*, reported on the morphology immunophenotype and genetic features of a large series of 42 PBL's cases from various anatomical locations. Their study highlighted the importance of *MYC* gene rearrangements (49%) mostly with the immunoglobulin heavy chain gene (*IGH*)⁵. This confirmed previous case reports and small series that suggest an important role for *MYC* in the pathogenesis of PBL^{3,9-12}. In addition, Valera and co-workers described frequent gains of *MYC*, *IGH* and other loci commonly involved in B-cell lymphoma⁵.

Despite these recent data, there is a significant shortage of genetic studies on this neoplasm likely due to its overall rarity, apart from countries with high incidences of HIV/AIDS. We recently published the clinico-pathologic, immunophenotypic and viral features of 45 PBL cases all affecting the oral mucosa¹ and we report here the genetic aberrations in what represents the largest series of PBL's affecting the oral mucosa to date.

Materials and methods

Case selection and clinical features

Forty-five cases confirmed as PBL using the WHO classification criteria², were retrieved from the files of the Departments of Oral Pathology at the Universities of Pretoria and Limpopo, South Africa. The clinical information and HIV status of all cases were obtained from the pathology request forms. The group consisted of 31 males, 12 females and 2 of unknown gender. Two patients were white males, 1 was of unknown race and the rest were black. The male to female ratio was 2,6:1. The mean age of the patients was 41years (29-58years). The HIV-status of 32 of the 45 patients was

known. Thirty one patients were HIV-positive and one was HIV negative. The morphology, immunophenotype and viral status were previously reported¹. In brief all cases had large blastic cells with various degrees of plasmacytic differentiation. Forty-four of 45 cases were EBV positive. Ki-67 and MUM1 were strongly positive in all cases while immunoreactivity for CD79a, CD38, CD138 and CD45 was variable. Light chain restriction was seen in 47% of these PBL cases¹.

Fluorescence in situ hybridisation (FISH)

Forty three cases were screened for breaks within the *MYC*, *IGH* and *BCL6* genes loci using Vysis LSI dual colour break apart (BA) rearrangement probes respective for each locus (Abbott Molecular Inc, IL, USA). The presence of *MYC/IGH* / t(8;14)(q24;q32), *CCND1/IGH* / t(11;14)(q13.3;q32) and *IGH/BCL2* / t(14;18)(q32;q21) translocations were detected with the Vysis LSI IgH/Myc:CEP 8 tri-colour, LSI *IgH/CCND1* dual colour dual fusion translocation and LSI *IGH/BCL2* dual colour, dual fusion translocation probes respectively. Four micron sections were deparaffinised, rinsed in absolute ethanol and air dried. After pre-treatment and digestion 10 µL of probe mixture (7 µL buffer solution, 2 µL distilled water, 1µL probe) was applied to the slides and coverslipped. Denaturation and overnight hybridisation was performed in a thermocycler (Thermobrite, Abbot Molecular Illinois, USA). Post hybridisation wash and counterstaining in 200 ng/ml 4', 6-Diamidino-2-phenylindole dihydrochloride (DAPI) (Merck, Darmstadt, Germany) were performed and slides were mounted with the Vectashield fluorescence mounting media (Vector Laboratories, Inc Burlingame, CA 94010). Results were evaluated with a Nikon Eclipse E1000 microscope equipped with a spectrum- green, orange, aqua, DAPI and triple band filters. FISH slides were screened by three independent analysts for verification of the results and selected images were captured using the Genus™ CytoVision 3.0 software (Applied Imaging). Slides were scored based on the knowledge of background levels of BA and fusion patterns in normal lymphoid tissue using at least 100 nuclei counts. Cut-off values for the FISH analysis were 5% for the BA probes and 15% for the dual fusion probes.

Results

FISH analysis

The combined results of FISH analysis, EBV positivity and HIV status are summarized in Table 1. *IGH* gene rearrangements were found in 27/43 (63%) of PBL cases screened with the *IGH* BA probe. Seven of these cases were found to have rearrangements affecting both *IGH* alleles.

Twenty-two of 43 cases (51%) were positive for the *MYC/IGH* translocation. The *MYC* dual-colour BA probe was used to determine if the *MYC* locus was rearranged in cases negative for t(8;14). Four of 20 cases (20%) screened showed positive BA signal of the *MYC* probes set and *MYC* was therefore rearranged in 26/43 (60%) of PBL's in this study with *IGH* a partner in 22 of the 26 cases (85%). The *CCND1/IGH* probe was hybridized to 41 specimens. The *CCND1* gene was found to be involved in a translocation with *IGH* in only one case, but interestingly, increased *CCND1* copy numbers were observed in 17 of 41 cases (41%) with 10 cases having three to five signals and seven cases having cells with more than six copies (Fig. 1). With the same probe set, six cases of PBL also showed gains of the *IGH* gene (Table 1).

Of the seven lymphomas with rearrangement of both *IGH* genes, two cases had a t(8;14) in association with a t(11;14) and a t(14;18) respectively; these were therefore double hit lymphomas. In three cases *MYC* was one of the *IGH* translocation partners while the other partner was not identified and both *IGH* chromosome partners remained unknown in two additional cases.

No rearrangements of *BCL6* gene could be detected in any of the 40 PBL's screened with the *BCL6* probe but gains with three to seven fusion signals per cell were observed in 11 cases (28%).

There was no correlation between the genetic features described here and the histomorphologic features such as the presence of plasmacytic differentiation. There was also no correlation between the immunohistochemical staining pattern for CD45, CD79a, CD138, CD38, MUM or the kappa and lambda light chains and the genetic features in any individual case of PBL included in this study.

Discussion

Thirty-one of the 32 patients (97%) with a known HIV-status in our study were HIV positive¹. In South Africa PBL is a neoplasm mostly related to HIV infection and we concur with others that the diagnosis of PBL should suggest immunosuppression in an individual with an unknown immune status^{10,13,14}. HIV-infected individuals are more prone to develop oral lymphomas which are heterogeneous in nature^{2,15,16}, more commonly associated with plasmacytic differentiation, occur at a younger age, have an aggressive clinical course with a poor prognosis and are frequently associated with other viral infections such as EBV¹⁷. These features were all observed in our patients.

Genetic features play a significant part in the 2008 WHO diagnostic algorithms for the classification of lymphoid tumours² and genetic data continue to inform on the pathogenesis of these diseases. Recurrent translocations of the *IGH* gene are commonly found in approximately 50% of all Non-Hodgkin B-cell lymphomas (NHL)^{18,19}. Rearrangements of *IGH* were observed in 27/43 (63%) of PBL cases in this series. In 82% of these (22 of 27 cases), the *MYC* gene was the *IGH* translocation partner. Four cases negative for t(8;14) also showed rearrangement of the *MYC* locus with partners other than *IGH*. *MYC* was therefore rearranged in 26 of 43 (60%) of PBL's in this study.

While *MYC* rearrangements are most often diagnostic for Burkitt's lymphoma (BL) they occur in other lymphomas²⁰ such as diffuse large B cell lymphoma (DLBCL) in which case they seem to be associated with an adverse prognosis²¹⁻²³. A high incidence of *MYC* rearrangements has been reported in several recent case reports and series of PBL^{3,5,9-12} and the median survival of patients varies from a few weeks to 3 years but seems to be reduced substantially when *MYC* rearrangements are present⁹⁻¹¹.

In the largest genetic study on PBL reported to date by Valera *et al.*, the incidence of *MYC* rearrangement was 49%. Their PBL cases were however from various anatomical locations and *MYC* rearrangements were more frequent in EBV-positive patients who represented less than 60% of their cohort⁵. Their series included eight cases of PBL of the oral mucosa, five of which (63%) had a *MYC* rearrangement and two cases with gains associated with *MYC*⁵. We observed *MYC* rearrangement in 60% of oral PBL in our series and 98% of these cases (44/45) were known to be EBV positive¹. EBV may thus be critical in the initiation of PBL of the oral cavity. The Waldeyer ring of lymphoid tissue, as well as the posterior oro-pharyngeal and naso-pharyngeal epithelial cells, is considered to act as EBV reservoirs²⁴. EBV reactivation during HIV infection might be explained by the decline of EBV-specific memory CD4+ and CD8+ T-cell responses²⁵. Of note, in our series, the only EBV-negative PBL was from a HIV negative patient, who also had no *MYC* or *IGH* rearrangements. The cooperation of EBV oncoviral proteins with the products of *MYC* gene aberrations may be the main pathogenic event for the development of lymphomas such as PBL and should be investigated further²⁶⁻²⁸.

Some mechanisms have been proposed to account for the aggressiveness of non-Burkitt's lymphoma with *MYC* gene rearrangements and the possibility that *MYC* may modulate different set of gene targets in non-BL when compared to BL was recently postulated²³. In addition *MYC* gene rearrangements in non BL are often associated with added cytogenetic abnormalities which may further contribute to the aggressive behaviour of the disease and to poor response to therapy observed in these patients^{5,12}. Seven PBL's cases in our series showed rearrangement of both *IGH* alleles. Both *IGH* translocation partners were unidentified in two cases, while *MYC* was one of the *IGH* partner in five cases. Among the latter, two cases showed involvement of the *CCND1* and *BCL2* genes respectively together with a *MYC/IGH* rearrangement. "Double hits" of *IGH* have been reported in several DLBCL and particularly in large B cell lymphoma with intermediate features between DLBCL and BL². To the best of our knowledge, the finding of rearrangement of both *IGH* alleles, although of low frequency (16%), is a new observation in PBL.

The *CCND1* gene was increased in copy number in 17/41 cases (41%), with more than six copies in seven of these cases. Cases with multiple *CCND1* copies suggest true amplification of this gene in some oral PBL. Cyclin D1 is a key cell cycle regulatory protein²⁹ and its increased gene copy number may drive uncontrolled cell proliferation and contribute to the high proliferation index and aggressive clinical course of PBL. *CCND1* gene amplification has been described in aggressive B-

cell NHL's³⁰ as well as in MM, in which case various mechanisms can be responsible for *CCND1* over-expression^{31,32}. To our knowledge *CCND1* amplification has not previously been described in PBL.

Although no rearrangements of *BCL6* were detected, gains of this gene were also observed in 11/40 (28%) of cases. Less than six copies were seen, likely to represent aneuploidy, further contributing to the complex cytogenetic features of these lymphomas.

Therapeutic approaches specific for *MYC*-associated lymphomas are lacking but bortezomib, a proteasome inhibitor widely used in the treatment of MM, is known to down regulate *MYC*³³. This might explain the recently reported dramatic response of a patient with PBL of the stomach to bortezomib treatment⁴. This study underlines the importance of routine evaluation of *MYC* rearrangements in PBL cases as this may have prognostic and treatment choice implications¹⁰.

There are no definite distinguishing features between PBL and MM or PBL and EMPC with plasmablastic morphology and the diagnostic boundaries between these neoplasms are sometimes blurred. Neither the patient HIV status nor the EBV positivity of the tumour helps to differentiate these neoplasms. Clinico-pathologic features of MM can be obscured in HIV/AIDS patients³⁴⁻³⁶ and cases described as PBL in the literature had clinical characteristics of MM such as monoclonal serum immunoglobulin³ and radiographically evident disseminated bone involvement^{9,35,37,38}. It is therefore clear that clinical features also do not serve as distinguishing features between these neoplasms. Although the high incidence of *IGH* rearrangement³⁹⁻⁴¹ and increased *CCND1* copy number echoes features of MM, the true relationship between these neoplasms should be investigated further.

In conclusion, PBL is a genetically complex neoplasm often associated with immunosuppression, particularly HIV/AIDS. We suggest that PBL of the oral cavity should be included as a group 1 lesion in the EC Clearinghouse and WHO Collaborating Centre classification of oral problems related to HIV infection⁴², especially in countries with high incidences of HIV such as South Africa. Our results, in line with others, reinforce the notion that EBV infection and *MYC* rearrangement are important events in the pathogenesis of oral PBL. Interestingly we have observed in our cohort rearrangements of both *IGH* alleles with two “double hit” cases as well as amplification of *CCND1* described in PBL for the first time.

Conflicts of interest statement

None declared.

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Table 1: *The table provides a summary of the EBV and HIV status as well as the FISH results obtained from this study.*

Case	HIV	EBV	IGH	MYC/IGH	MYC	CCND1/IGH	BCL6	IGH/BCL2
1	+	+	ND	ND	ND	ND	ND	ND
2	+	+	R	R	ND	-	-	ND
3	+	+	R	R	ND	-	-	ND
4	+	+	R / DR	R	ND	G (11 & 14)	ND	-
5	+	+	R	-	-	-	G	ND
6	+	+	-	-	-	-	ND	ND
7	+	+	R	R	ND	G (11)	G	ND
8	U	+	-	-	-	G (11)	-	ND
9	+	+	R	R	ND	G (11)	-	ND
10	+	+	R	R	ND	-	-	ND
11	+	+	R	-	R	G (11)	-	ND
12	+	+	R	R	ND	-	-	ND
13	+	+	-	-	ND	-	-	ND
14	+	+	R / DR	-	-	G (11)	G	-
15	+	+	R / DR	R	ND	-	-	-
16	+	+	R / DR	-	R	G (11 & 14)	G	-
17	-	-	-	-	-	G (11)	-	ND
18	+	+	-	-	-	G (11)	-	ND
19	+	+	R	R	ND	-	ND	ND
20	U	+	-	-	-	G (11)	-	ND
21	U	+	R	R	ND	G (11)	G	ND
22	U	+	R	R	ND	-	-	ND
23	U	+	-	-	-	ND	-	ND
24	U	+	-	-	-	-	-	ND
25	U	+	R	R	ND	-	L	ND
26	U	+	R	R	ND	-	-	ND
27	U	+	R / DR	R	ND	R/G (11 & 14)	-	-
28	U	+	R	R	ND	-	-	ND
29	+	+	R / DR	R	ND	G (11 & 14)	G	-
30	U	+	-	-	-	-	-	ND
31	+	+	-	-	-	-	-	ND
32	+	+	R	-	-	-	G	ND
33	+	+	R	R	ND	G (11 & 14)	-	ND
34	+	+	-	-	-	-	-	ND
35	+	+	-	-	R	-	L	ND
36	+	+	R	R	ND	-	G	ND
37	U	+	-	-	R	-	-	ND
38	+	+	R	R	ND	G (11 & 14)	G	ND
39	+	+	-	-	-	-	G	ND
40	U	+	ND	ND	ND	ND	ND	ND
41	+	+	-	-	-	G (11)	-	ND
42	+	+	-	-	-	G (11)	G	ND
43	+	+	R	R	ND	-	L	ND
44	+	+	R	R	ND	ND	-	ND
45	+	+	R / DR	R	ND	-	-	+

R = positive for rearrangement; - = negative; ND = not done; DR = double rearrangement; G = gain; G (11) = gain of CCND1 on chromosome 11; G (11 & 14) = gain of CCND1 on chromosome 11 and of IGH on chromosome 14; G (14) = gain of IGH on chromosome 14; L = loss

Figure 1

DAPI stained interphase nuclei of a PBL case hybridised with the IGH/CCND1 dual colour dual fusion translocation probe (Vysis®, Abbot Laboratories). Four to ten copies of the CCND1 gene on chromosome 11 are represented by the orange signals (red arrows). Green signals represent the IGH gene on chromosome 14 which also shows multiple copies per nucleus (green arrow). Note that the IGH gene was also rearranged on the IGH BA probe analysis of this case.