

Immunostaining by Human Herpes Virus 8 Latent Nuclear Antigen-1 of Kaposi's sarcoma: A Potential Biomarker of Severity of Disease?

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Abstract

Objective: To investigate the clinical relevance of scoring Kaposi's sarcoma herpes virus (KSHV) expression by Human Herpes Virus-8 (HHV-8) latency-associated nuclear antigen 1 (LANA 1) in the early (early patch and plaque) and late (nodular) stages.

Methods: We applied a combined intensity weighted histoscore and a categorized score to 235 early patch, plaque and nodular stages, and compared our results with published data.

Results: The mean individual scores were significantly lower in the early patch stage compared to the plaque and nodular stages. There was no significant difference in the mean individual scores between the plaque and nodular stage. There was a wide overlap in distribution of histoscore between the three stages.

Conclusion: Since treatment modalities are not based on the histological stage nor on the histoscore, KSHV histoscore appears not to be a useful biomarker of the severity of the disease.

Keywords: Kaposi's sarcoma; HHV-8; LANA-1; Immunohistochemistry; Histoscore; Biomarker

Impact: KSHV score is not a potential biomarker of severity of Kaposi's sarcoma.

Introduction

Chang et al. were the first to identify herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma (KS) [1]. It is now well documented that the human herpes virus 8 (HHV-8) or Kaposi sarcoma-associated herpes virus (KSHV) is the causative agent of KS in all its forms (classic, epidemic, endemic, iatrogenic immunosuppression). Initially, the detection of HHV-8 DNA or messenger RNA was carried out by a polymerase chain reaction (PCR) based technique, a highly sensitive test for KSHV [2]. Arguably, it is even too sensitive for the differential diagnosis of non-KS vascular lesions, since some of them have been shown to be HHV-8 PCR positive. For this reason, HHV-8 immunohistochemical expression on formalin-fixed and paraffin-embedded tissue has become the gold standard for the diagnosis of KS.

Dupin et al. were the first to carry out immunohistochemistry with a monoclonal antibody directed against the HHV-8 latency-associated nuclear antigen 1 (LANA-1) [3]. LANA-1 is a latent specific gene that promotes cell cycle progression through competition with E2F for the retinoblastoma protein (pRb), and decreases apoptosis through interaction with p53 [4-6]. Since the early 2000s, LANA-1 immunohistochemistry has become the gold standard for the diagnosis of KS [4,7].

KS progresses through subsequent stages from the early patch, through the plaque and nodular stages [8]. Earlier studies have demonstrated that this progression parallels an increasing number of HHV-8 labeled cell nuclei [2,3,5,6,9-12]. These studies involved a relatively low number of cases (between 12 and 50), perhaps too small to reach statistical significance [3,10].

Traditionally and for descriptive convenience, the histological spectrum is divided into four subgroups; in reality, however, there is overlap between stages and there are no differences in the pathology of the disease in the different risk groups (i.e. classic, epidemic, endemic,

iatrogenic immunosuppression). Therefore, it may be artificial to subdivide KS in early (i.e. early patch and plaque stages) and late (nodular stage) stages. Moreover, the aggressive late stage variant, the fourth subgroup, is said to be extremely rare [8].

The aim of the present study was to submit a large series of consecutive KS to a histoscore combining the staining intensity and the percentage of LANA-1 labeled cell nuclei as proposed by Cheuk et al. [10] in order to validate the method as a potential biomarker of the severity of KS.

Materials and Methods

The study was carried out at the Histopathology Department of the National Health Laboratory Service in Polokwane, Limpopo. A prospective series of 235 biopsy diagnosed KS confirmed by HHV-8 immunostaining entered the study. The cases were collected between September 2011 and August 2012, and were studied anonymously. Ethical approval was obtained from the Polokwane/Mankweng Hospital Complex and the University of Limpopo research ethics committee.

The specimens were fixed in 10% phosphate buffered formalin and embedded in paraffin. Four micrometer (μm) sections were stained with Hematoxylin and Eosin for initial diagnosis. All of the cases were routinely streptavidin-biotin-peroxidase immunostained with diaminobenzidine using a murine monoclonal antibody directed against the C-terminus of the LANA-1 molecule of HHV-8 (clone

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13b10; Novocastra, New Castle upon Tyne, UK). The dilution was of 1:100. The incubation time was 30 minutes at room temperature.

The subdivision into early, plaque, and nodular stages is based on their mode of progression [9]. Early patch stage is diagnosed by the presence of scattered dilated irregular or angulated lymphatic-like spaces lined by delicate, bland endothelial cells. A diagnosis of plaque stage is reached in the presence of a band of spindle cells, slit-like vascular spaces and interstitial hemorrhages. The nodular stage is characterized by nodules (instead of plaques) of spindle cells mixed with vascular spaces [8]. Our material comprised 101 early stages, 47 plaque, and 87 nodular stages.

A semi-quantitative histoscore was implemented combining staining intensity (1, weak; 2, moderate; 3, strong) and the percentage of labeled nuclei (1, 1-25%; 2, 26-50%; 3, 51-75%; 4, 76-100%), as proposed by Cheuk et al. [10]. We also counted the number of stained nuclei per high power field (HPF 10 × 40).

Statistical analysis was carried out with the GraphPad software from Prism (San Diego, CA). Column statistics, Student's *t* test, and 95% confidence intervals of proportions were used. The level of statistical significance was set at *p*<0.05.

Results

There were 101 (43.0%) early patch, 47 (20.0%) plaque, and 87 (37.0%) nodular stages. Table 1 shows the mean scores of four individual parameters for the early patch, plaque, and nodular. The individual scores and total score of early patch stages were significantly lower than corresponding scores of plaque stages. The only statistically significant difference between nodular and plaque stages was the percentage of stained nuclei per high power field (*p*=0.042).

Table 2 illustrates the distribution of the total histoscore by stage. There was an overlap of histoscore of the different stages since all stages were represented in each of the categories. There was a trend towards lower scores (< 4) in the early patch stage (80/101, or 79.2%) and a weak but inconclusive trend towards higher scores (> 5) in the plaque (24/47, or 51.0%) and nodular (52/87, or 59.8%) stage.

Discussion

From the time of the first description of KS the condition has been shrouded in ambiguity. Interestingly, Moritz Kohn, who described the first classic cases, dropped his surname to adopt the name Kaposi after his birthplace in Hungary, Kaposivar on the Kapos River [13]. Since

then the condition has kept its original qualification (i.e. sarcoma) although certain current writers call it a disease, a lesion, a potentially life-threatening neoplasm, or cancer [13-18]. Others hold the view that it is unclear whether KS is a reactive process or a true malignant neoplasm [8,19]. The qualifiers attributed to KS vary from "a highly vascularised tumour-like lesion" to "a vasoformative tumour of uncertain origin and complex pathogenesis" [5,6,20,21].

Although KSHV is recognized as the causative agent of all types of KS, the natural history of classical and endemic KS differs significantly from the AIDS-related epidemic KS. The cell of origin has been widely investigated; most studies have suggested a vascular endothelial, a lymphatic endothelial, a precursor endothelial cells, or a dermal dendrocyte [3,5,6,14,19,21,22]. A lymphatic endothelial precursor cell origin is most likely [5].

Both LANA-1 and lymphatic marker D2-40 expression have been reported to increase with tumour progression [12,22]. Therefore, it has been suggested that they could be used as tissue biomarkers in defining patients with a higher risk of disease progression [11]. According to Dupin et al. [3], LANA-1 is expressed respectively in <10, up to 50, and >90% of spindle cells in early patch, plaque, and nodular stages respectively. Cheuk et al. [10] found 10-20% LANA-1 expression in the early stages (early patch and plaque), and 70-80% in the late nodular stage; however, they did not assess the statistical significance of the distribution of the respective scores by stage nor did they comment on the possible usefulness of their semi-quantitative scoring system as a potential biomarker of severity of the disease. Our statistical evaluation of their cases showed no difference between the scores for early patch and plaque stages (*t*=0.1; *p*=0.90). Our data showed no difference in histoscore between the nodular and the plaque stages.

It has been shown that the prognostic determinants of AIDS-KS are tumour extension and systemic disease [16]. Patients with AIDS-KS have more opportunistic infections and higher mortality rates, but lower median viral loads than endemic KS [23,24]. Therefore, one could speculate that the high LANA-1 histoscore in nodular stage KS does not as such express a poor prognosis.

Treatment of KS is systemic in disseminated disease, and local for focal single or few lesions [25-27]. Although highly active antiretroviral therapy (HAART) has reduced the incidence of AIDS-KS, only half of the cases achieve resolution; moreover, HAART and other therapies (i.e. radiotherapy and chemotherapy) do not prevent new KS lesions from developing [28]. Prognostic indexes for AIDS-associated KS in the era of HAART have used having KS as the AIDS-defining illness,

Parameter	Early patch n=101	Plaque n=47	Early vs. plaque		Nodular n=87	Nodular vs. plaque	
			t	p		t	p
Nb stained nuclei/HPF	41.6 (63.4) [18.0]*	162.0 (221.4) [72.5]	5.0	<0.0001	232.5 (228.1) [170.0]	1.7	0.087
% stained nuclei/HPF	21 (22.2) [10.0]	37.9 (30.2) [40.0]	5.0	<0.0001	49.0 (29.6) [50.0]	2.1	0.042
Staining intensity	1.8 (0.8) [2.0]	2.2 (0.8) [2.0]	2.8	0.005	2.4 (0.8) [3.0]	1.4	0.17
Total score	3.6 (1.5) [3.0]	4.5 (1.9) [5.5]	3.1	0.002	5.1 (1.8) [5.0]	1.8	0.07

*Values are mean (SD) [Median]

Table 1: Mean scores of individual parameters according to histopathological stage.

Stage	N cases	2	3	4	5	6	7	Mean score
Early patch	101 (43.0)	24 (23.8)	30 (29.7)	26 (25.8)	9 (8.9)	5 (4.9)	7 (6.9)	3.6 (1.5) [3.0]
Plaque	47 (20.0)	6 (12.8)	6 (12.8)	11 (23.4)	5 (10.6)	8 (17.0)	11 (23.4)	4.5 (1.9) [5.5]
Nodular	87 (37.0)	13 (14.9)	7 (8.0)	15 (17.2)	10 (11.5)	16 (18.4)	26 (29.9)	5.1 (1.8) [5.0]
Total	235	43 (18.3)	43 (18.3)	52 (22.1)	24 (10.2)	29 (12.3)	44 (18.7)	

Table 2: Relative distribution by stages' total histoscore.

having another AIDS-defining illness, CD4+ T-cell counts, and age as prognostic factors [29]. Others used staging at the time of diagnosis, low CD4, and HHV-8 viral load to predict the evolution and the need for chemotherapy [28]. To the best of our knowledge, the histological stage (i.e. early vs. late) is not used to determine the therapeutic modalities or the prognosis.

In conclusion, there is overlap between the relative distribution of individual scores of early and late stages of KS. The present findings do not support the use of HHV-8 LANA-1 histoscore as a potential biomarker of progression of KS.

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