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Kaposi's Sarcoma in The Democratic Republic of Congo: Immunohisto-Chemical Expression and Proliferative Activity in Different Histological Types

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Kaposi's sarcoma; Democratic Republic of Congo; Immunohistochemistry; Proliferative activity

1. Abstract

In a preceding study, Kaposi sarcoma (KS) associated with human immunodeficiency virus (HIV) has been found frequent in the Democratic Republic of the Congo (DRC).

1.1. Aim

To confirm the previous clinical and histological diagnosis of KS in the DRC on specimens of biopsies sent to Belgium by immunohistochemistry.

1.2. Materials and Methods

On 142 specimens from patients with the presumed diagnosis of KS in the DRC and 48 specimens from DRC patients with various vascular lesions, identification of the HHV-8 virus with the anti-HHV-8 antibody and of the vascular and lymphatic endothelial cells with the anti-CD31 and anti- D34 antibodies, were performed as well as the determination of the proliferative activity of KS tumor cells by the Ki-67 antibodies.

1.3. Results

The diagnosis of KS was confirmed by the immunohistochemical identification of the HHV-8 virus in all the specimens from patients with the presumed diagnosis of KS in the DRC and in the specimens of 6 patients with benign vascular lesions. The highest proliferative Ki-67 index was found in the patients of the KS sarcomatous type.

1.4. Conclusions

The identification of the HHV-8 virus is the best mean for the diagnostic of Kaposi's sarcoma, particularly in atypical cases.

2. Introduction

In the Democratic Republic of the Congo (DRC) the epidemic variant of Kaposi's sarcoma (KS) was predominant over the sporadic variant, following a recent study [1]. The diagnosis of KS in the DRC was previously based solely on a clinical basis and on biopsies stained with hematoxylin-eosin (HE), without the identification of the causal agent, the Human Herpes Virus 8 (HHV-8). The aims of the present study performed on specimens from biopsies taken in patients with a presumed diagnosis of KS in the DRC were:

- to evaluate the specificity of the HE staining for the diagnosis of KS
- To identify the presence of HHV-8 in the lesions of presumed KS.
- to quantify the proliferation of vascular endothelial cells, lym-

phatic endothelial cells and tumoral cells infected by HHV-8

- To search for stigmata of inflammation on the biopsies analyzed by histochemical methods.

3. Materials and Methods

KS biopsies collected by surgeons and/or dermatologists during the period 2000-2016 from different hospitals of the DRC were fixed in 10% formalin and embedded in paraffin blocks, then cut in thin sections (5 μm) for staining with HE. The first histological diagnoses in the DRC have been cross-checked in the Pathology laboratories of the St Luc University Clinics of Louvain (UCL, Brussels/Belgium). Glass slides with a presumed KS diagnosis were reviewed by 2 pathologists to confirm the diagnosis. The remaining materials were analyzed by immunohistochemical (IHC) staining. Specific primary antibody staining was detected using Envision (Dako, North America) with diaminobenzidine tetrahydrochloride as chromogen with the use of a benchmark XT automaton (Ventana-Roche, Tucson-USA). The anti-HHV-8 antibody permitted to establish the viral diagnosis of KS. while the CD31 and CD34 antibodies evaluated the proliferation of vascular and lymph endothelial cells. Finally, the proliferative activity of the tumor cells infected by HHV8 was quantified by the Ki-67 antibody. A percentage of better than 5% of cells stained with the Ki-67 antibody was considered as significant of a proliferation of tumor cells, using a semi-quantitative method. Negative controls (n =48) included specimens from patients with various benign vascular lesions diagnosed in the DRC, predominantly hemangiomas, lymphangiomas and pyogenic granulomas. Desmin and actin staining were used to exclude a tumor of muscular origin. Positive controls (n= 28) were KS patients HIV (+) (n=10), HIV (-) (n=5) and unknown HIV status (n=13), followed at the St Luc University Hospital (UCL) during the period 2011 to 2014

The histological classification of the stages (types) of KS lesions following the disease progression proposed by W. Grayson and Pantanowitz (2008), 4with illustrative photographies was used:

1. Patch stage (also named angiomatous);slit-like vascular spaces; erythrocyte extravasation; infiltrate of lymphocytes and plasma cells.

2. Plaque stage (also named mixed); more diffuse vascular infiltrate; spindle lesional cells; dissecting vascular channels containing erythrocytes, plasma cell-rich contingent of chronic inflammatory cells.

3. Nodular Kaposi's sarcoma: dermis expanded by a proliferation of neoplastic spindled cells.

4. Statistical Analyzes

They were performed using Chi-square tests. A P-value < 0.05 was considered as significant to reject the null hypothesis.

4.4. Ethics

The study was approved by the Ethical Committee of the Faculty of Medicine of the Catholic University of

Bukavu (DRC)

5. Results

From the194 patients, whose histological diagnosis of KS was presumed by HE staining in the DRC during the period 2000 to 2016, we analyzed the specimens of 142 patients brought to the UCL Pathology Laboratory, as well 48 specimens from patients with various vascular lesions. The results of HHV-8 tagging with the anti-HHV-8 antibody, anti-CD31 and anti-CD34 antibodies are indicated in Table 1. Biopsies taken in the DRC with a KS diagnosis based on HE staining and confirmed by the anti-HHV-8 antibody, originated from 135 patients with epidemic KS and 7 patients with sporadic KS. Histological types of KS were of the angiomatous type (stage1) corresponding to an early stage in 25/142 specimens (17.6%), of the mixed type in 23/142 specimens (16.2%) corresponding to a mixture of vascular structures and fusiform cells and of the nodular and/or sarcomatous type corresponding to the late stage or stage 3 in88/142 specimens (61.9%). Other KS diagnoses revealed by HHV-8 immunostaining were found in the negative control group in 6 cases (4.2%), including pyogenic granuloma, lymphangioma, and hemangioendothelioma, initially considered by the HE staining as benign vascular lesions. The comparison between the results of HE staining and the results of immunohistochemical staining were summarized in Tables 1 and 2. There were not statistically significant difference between the results of both method (P-value=0.98). The results of the studies comparing the proliferative activity index (Ki-67) of the tumours

in function of the histological types are presented in the Table 3. In the cases of the sarcomatous KS (stage 3) there were more cases (n=65) with a proliferative index (Ki-67) higher than 41%. The results were significant than in the other KS types (n = 6), the Ki-67 median was 13.5%. In addition, the median Ki-67 at P-value in the 7 cases of endemic KS was cases 26% and the result was not significant at P-value 0.05 (P-value = 0.84).

Table 1: Results of immunohistochemistry in patients with the presumed diagnosis of KS in the DRC and in the control groups.

Groups of Patients	HE staining in the DRC	HE staining in St Luc UCL	Anti-HHV-8 positive	Anti-CD31 positive	Anti-CD34 positive
Patients with presumed KS diagnosed in the DRC	142	142	142 (100 %)	127 (89.4%)	128 (90.1%)
Benign vascular lesions from the DRC	48	48	6(12.5%)	46 (95.8 %)	42 (87.5%)
Control group of Belgian patients with KS		28	28	17 (60.7%)	15 (53.6%)

Table 2: Histological types of KS compared to HHV-8, CD31 and CD34 positive immunostainingin 142 patients.

Histological types of KS	Positive Immunohistochemical staining		
HE staining N = 142	Anti-HHV-8 N = 142	Anti-CD31 N = 132	Anti-CD34 N = 131
1. Angiomatous	25 (17.6%)	25 (18.9%)	23 (17.5%)
2. Mixed	23 (16.1%)	21 (15.9%)	20 (15.2%)
3. Sarcomatous	88 (61.9%)	81 (61.3%)	85 (64.8%)
Others KS types	6 (4.2%)	5 (3.6%)	3 (2.2%)

P-value = 0.9824.

Table 3: Ki-67 proliferation index in different histological types in 135 AIDS-related KS cases.

HISTOLOGICAL TYPES	Ki-67 PROLIFERATION INDEX (%)			Number of patients N = 135
	5-20 %	21-40 %	> 41 %	
Angiomatous	10	8	7	25
Mixed	3	13	3	19
Sarcomatous	11	8	65	84
Others KS types	4	1	1	6

P-value < 0.001.

Figure 1: Microphotographs of some cases finding of KS at different stages associating HE and IHC for some patients.

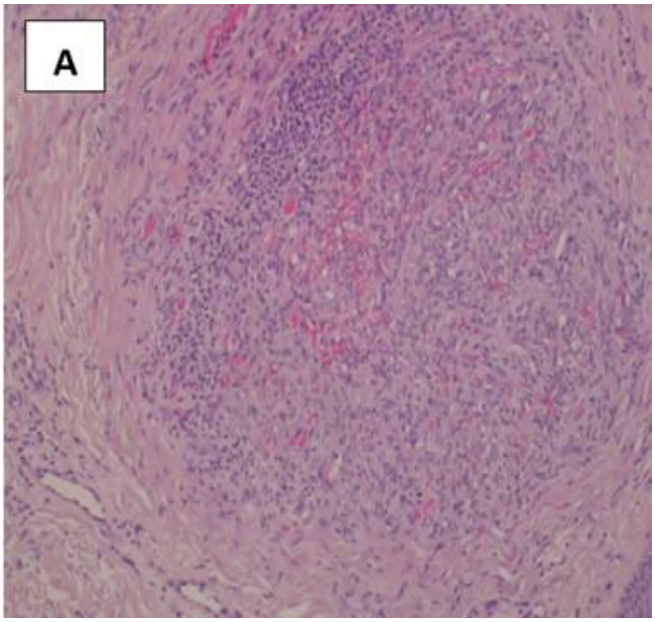

Figure 1A: KS sarcomatous type (H & E, x 12,5) in 48-year-old man from the Eastern part of the DRC with disseminated nodular lesions. Histologically: Focused or diffuse proliferation of the spindle cells, which are most often arranged in fascicular and nodular structures with an abundant inflammatory infiltrate.

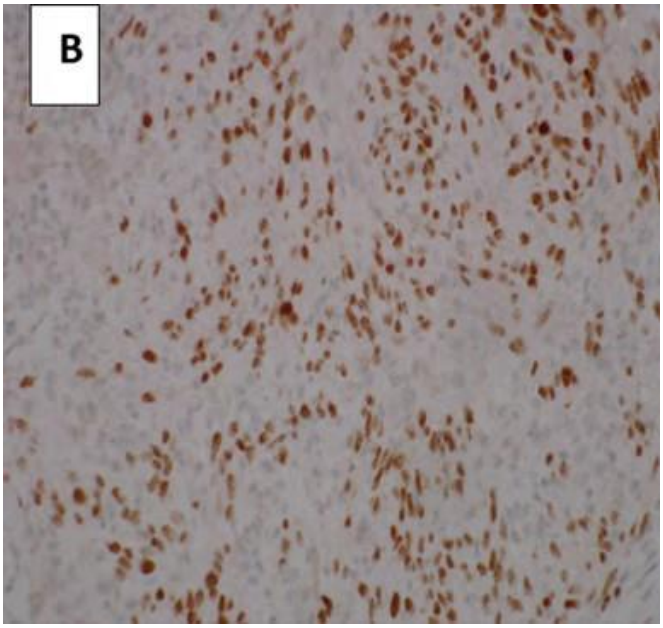

Figure 1B: HHV-8 staining, x 25 Positivity for the HHV-8 nuclear antigen was observed in fusiform and endothelial cells in advanced KS lesion.

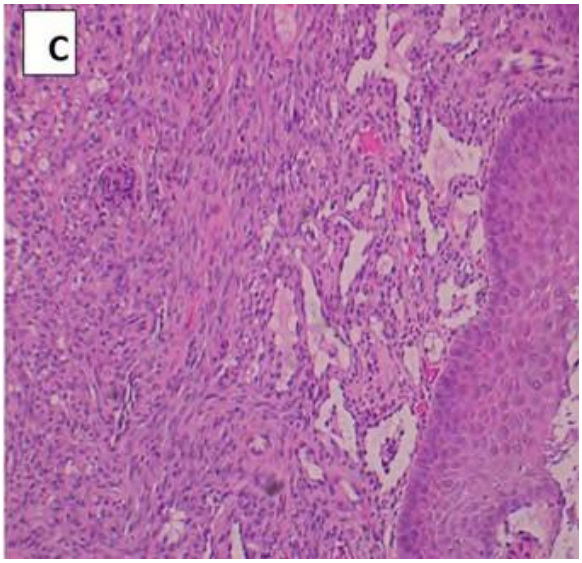

Figure 1C: KS mixed type (H & E, x 20) in a 23-year-old man from the Western part of DRC, infected by HIV with plaque skin lesions. The histological lesions were characterized by a mixed proliferation associating diffuse spindle cellularity and vascular structures. The inflammatory infiltrate is discreet.

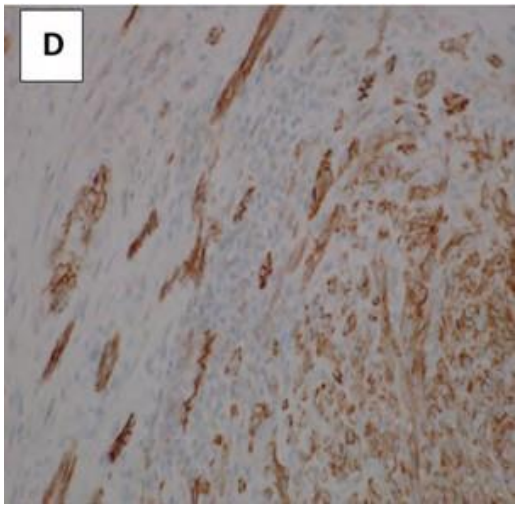

Figure 1D: CD31 staining, x 20 A positive immune reaction with CD31 was evident in endothelial cells.

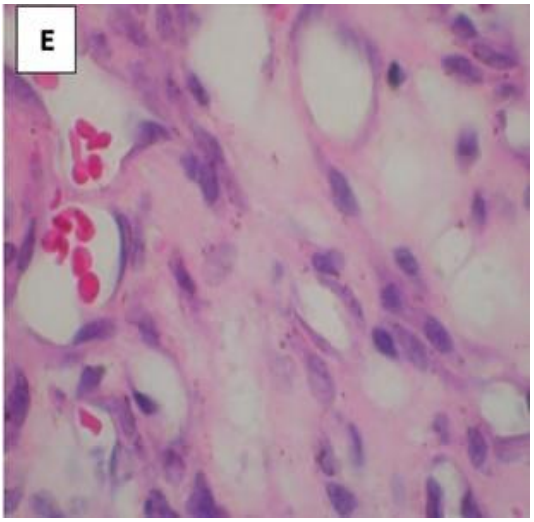

Figure 1E: KS angiomatous type (HE staining, x 20) in 36-year-old women from the Western part of the DRC. Histologically, the vascular proliferation is predominant with a discreet inflammatory infiltrate.

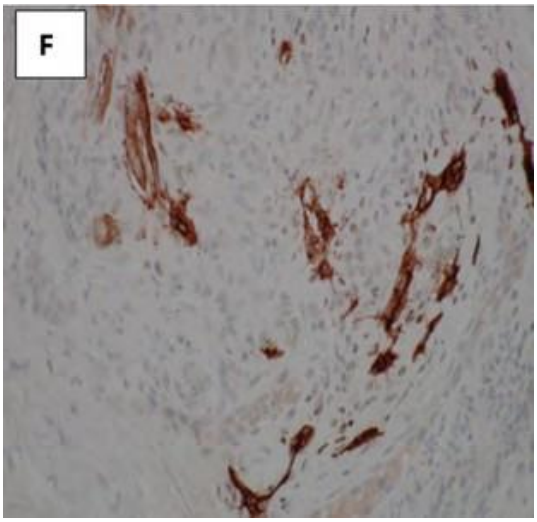

Figure 1F: CD34 staining. A positive immune reaction with CD34 was evident in endothelial cells.

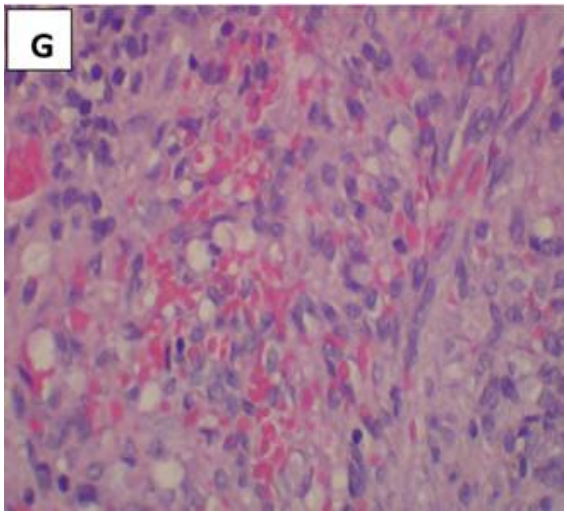

Figure 1G: KS sarcomatous type in a 25-year-old man from the Eastern part of DRC with florid ulcerative lesions. A mitosis at metaphase is shown, together with hemorrhagic suffusions and moderate inflammatory infiltrate. H & E staining. H &E, x 40.

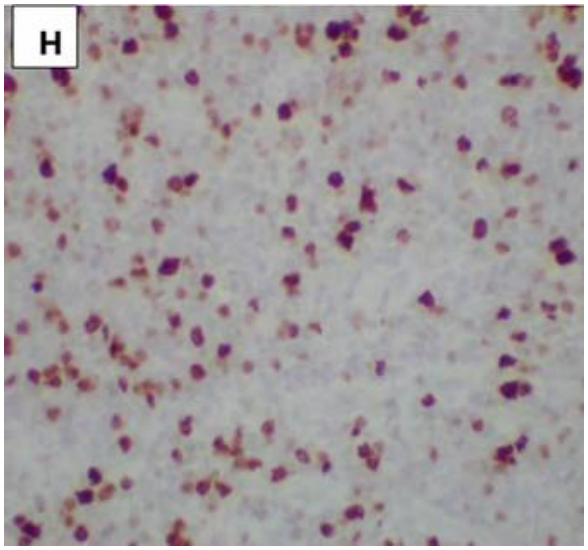

Figure 1H: Ki-67 staining, x 40 There was a positive immune expression of Ki-67 in nuclear of spindle cells.

6. Discussion

6.1. Histological Features

Our study revealed the existence of three progressive histological stages of KS, with a predominance of the stage 3of nodular and/or sarcomatous stage in the DRC. Different morphological aspects of KS were reported. Therefore, it is indicated to recognize the unusual forms in order to avoid potential misdiagnosis and an inappropriate management of afflicted patients. According to several authors(1-3), there has been an increasing awareness of a wider clinico-histological spectrum of KS including lymphangioma KS, lymphangiectactic KS, telangiectatic KS, bullous KS, vesicular KS, micronodular KS, keloidal KS, hyperkeratotic KS, pyogenic granuloma-like KS, ecchymotic KS, regressed KS lesion and anaplastic KS. This is why HHV-8 recognition by the HHV-8 antibody allows to confirm the diagnosis of KS .

6.2. Immunohistochemical (IHC) Study

Using the anti-HHV-8 antibody, the confirmation of KS diagnosis was made possible in all our cases of KS and of some KS cases discovered in the control group of vascular lesions. The CD31 and CD34 markers were expressed in the majority of the lesions that contained endothelial cells. The HHV-8 immunohistochemical detection is an effective tool for KS diagnosis, especially in KS early lesions (eg. Stage 1) [1] and when neoplastic features are not evident such as in other rare lesions as dermatofibroma, hemangioma, pyogenic granulosa [4,5].

The origin of the spindle cells involved in the pathogenesis of KS was made possible by immunohistochemistry [6]. According to several authors [7], the various histological stages can be detected by CD31 and CD34 endothelial markers, whatever the clinical type of the disease. Hence their choice in this study. For other authors, the marker that best applies to the vascular endothelium would be the CD31 compared to CD34 which reacts remarkably on the endothelium of the lymphatic capillaries. Although, it is still controversial [8,9,7]. Stated that there was no link between HIV immunosuppression and immunoblotting of cells infected with HHV-8 and added that it was no significant the IHC staining difference observed between clinical subtypes and tumor stages of KS (nodular, patch) [1]. Recommended the use of the antibody to HHV-8 LNA-1, as well as the lymphatic endothelial cell marker D2-D4O Instead of the less specific vascular markers CD31 and CD34.

6.2. Proliferative Marker (Ki-67)

In the literature [10], the results of the studies comparing the different clinical stages and the proliferation activities of the KS are controversial. They investigated whether there is a correlation between the proliferation activities of the skin and those of the internal organ lesions in 13 cases of KS. They found that the proliferation activities of KS did not differ significantly.

In a Israel study on histopathology of 45 KS cases including 26 classic KS and 19 iatrogenic KS, the authors(11) reported the predominance of all histological types including angiomatous KS as the early stage, mixed KS and KS with spindle cells as the late stage. They observed that the progression of the tumor was more in favor of proliferation of spindle cells than proliferation of endothelial cells. They concluded that the proliferative activity (Ki-67) of the cells infected with HHV-8 was higher in the classic KS than in iatrogenic KS. In a series of 78 Tanzanian patients with oral KS studied from 1990 to 2005, there were more epidemic KS (74/78) than endemic KS (4/78). The histopathological diagnosis was predominantly nodular, with fusiform cells. Immunostaining demonstrated the existence in adult males of numerous proliferating cells with an elevated Ki-67 index (median = 24.1%) compared to females (17.2 %). A correlation was found between the proliferation index (Ki-67) and the high content of HHV-8 [12]. Other studies

did not confirm our observations. In a study in Sweden [13] on biopsies of epidemic KS (n=22) and endemic KS (n=7) the authors concluded that the Ki-67 index in all clinical stages (early and late) ranged between 4.5 and 11.5%. Our results showed, like those of this study, a proliferative advantage for the spindle cells KS compared to other histological types.

7. Conclusion

Some diagnoses of KS especially at its early stage or of the angiomatous type as well as some benign vascular lesions cannot be confirmed by the HE staining, Hence the immunohistochemistry using HE and- HHV-8 antibody is useful. The expression of Ki-67 in cells infected by HHV-8 were high in AIDS-related KS lesions, particularly the histological sarcomatous type.

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