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Tumor immune microenvironment (TIME) in BPV1-positive equine sarcoids

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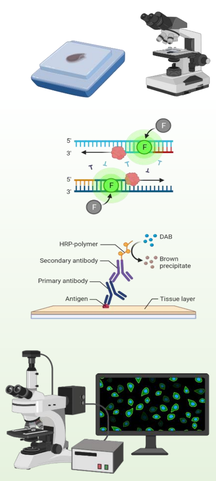
BACKGROUND & AIM

- Equine sarcoid is the most commonly diagnosed cutaneous tumor in horses, representing up to 90% of all skin tumors [1].
- Bovine Papillomaviruses (BPVs) type 1, and less commonly 2, are likely involved in tumor pathogenesis [2].
- The tumor immune microenvironment (TIME) promotes the uncontrolled replication of BPV within the infected fibroblasts.
- Despite the many efforts made for the development of effective therapy, there is still no treatment recognized as universally successful.
- The purpose of the study is to give a preliminary insight into TIME of equine sarcoids, aiming at a future identification of prognostic factors or therapeutic targets

MATERIALS & METHODS



Thirty-five equine sarcoid samples were retrospectively selected and histopathologically re-evaluated.

BPV1-DNA was detected by real time quantitative (q)PCR and *in situ* hybridization (ISH) was performed to assess E6/E7 expression.

Expression of immune-related markers CD3, CD20, Iba1, CD204, CD163, and MUM1 was quantitatively assessed through immunohistochemistry (IHC).

Double immunofluorescence (IF) was performed on selected cases, to evaluate colocalization of immune markers.

Images from "Materials & methods" section created in BioRender.com

RESULTS

All selected samples were diagnosed as sarcoids (**Fig. 1A and 1B**) and resulted positive for BPV1-DNA, while 24/35 (68.57%) expressed E6/E7 oncogenes on ISH (**Fig. 1C and 1D**).

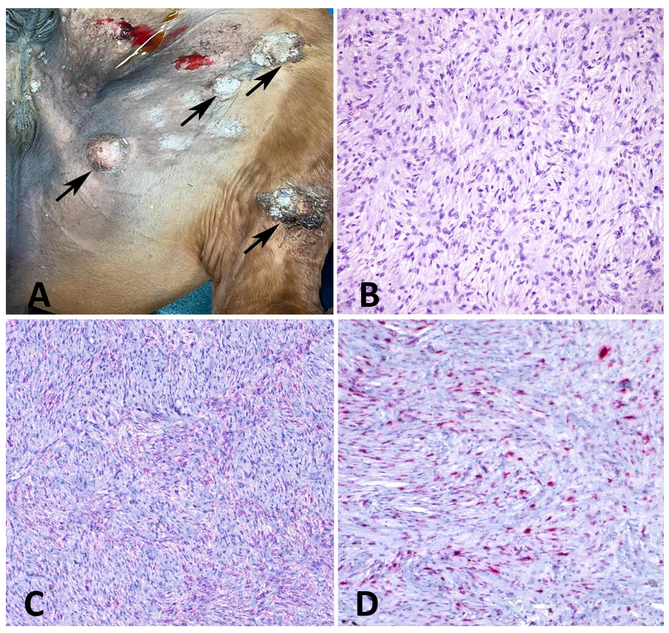


Figure 1: (A) Nodular sarcoids, horse. These lesions are characteristically slow growing, usually progressing to "warty" verrucous growths or developing rapidly into fibroblastic lesions. (B) Equine sarcoid. Hematoxylin and eosin (H&E). Microscopically sarcoids might appear as areas of high cellularity characterized by spindle to stellate cells embedded in finely fibrillar to dense collagenous stroma and organized in parallel and perpendicular rows. (C) Equine sarcoid. ISH. The labelling is represented by magenta dots located in the cytoplasm of neoplastic fibroblasts. (D) Equine Sarcoid. ISH. Dots disseminated within the nuclei of neoplastic cells.

IHC showed that most tumors were immune-deserted or excluded (71.43%), based on the presence of CD3⁺ and CD20⁺ tumor infiltrating lymphocytes (TILs). Intratumorally, the immune infiltrate was mostly composed of CD163⁺, CD204⁺, and Iba1⁺ macrophages (**Fig. 2**).

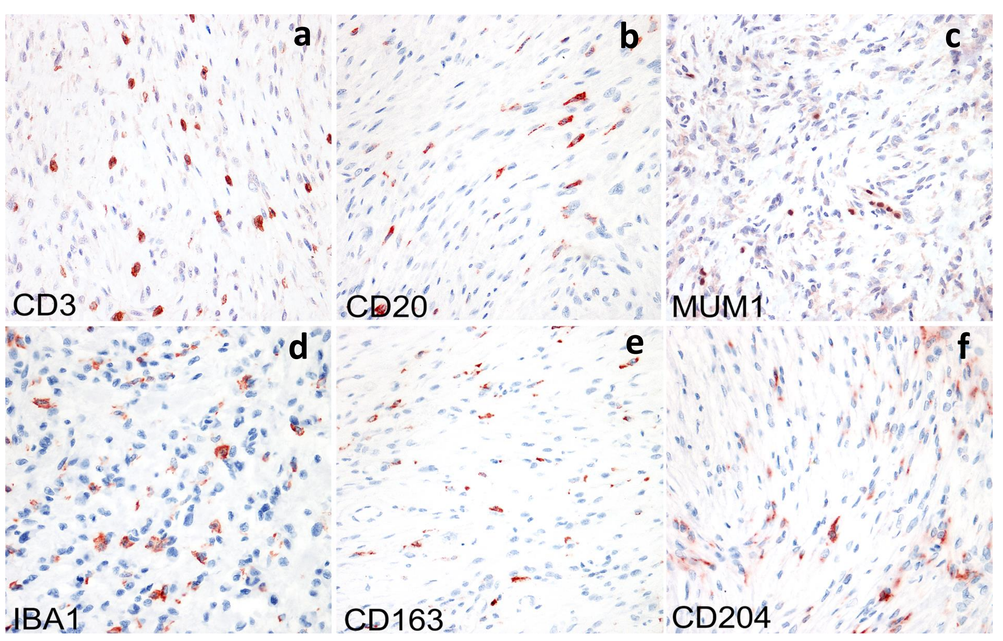


Figure 2: IHC of CD3⁺(a), CD20⁺(b), MUM-1⁺ (c), Iba1⁺ (d), CD163⁺ (e) CD204⁺ (f); (a) Equine sarcoid. The number of CD3⁺ lymphocytes infiltrating the tumor was low, with cells mostly arranged individually within the tumoral stroma. (b) Equine sarcoid. Few CD20⁺ B cells were observed in direct contact with tumor cells. (c) Equine sarcoid. The number of MUM1⁺ cells was low within the neoplastic areas; only occasional infiltrates could be seen peritumorally. (d) Equine sarcoid. The number of Iba⁺ cells were often seen infiltrating among tumor cells. (e) Equine sarcoid. CD163⁺ cells are often scattered among tumor cells. (f) Equine sarcoid. CD204⁺ cells were often observed within the tumoral stroma.

The number of tumor infiltrating MUM1⁺ plasma cells and CD20⁺ lymphocytes was lower compared to CD163⁺, CD204⁺ and Iba1⁺ macrophages (**Fig. 2; Fig. 3**). Spearman's correlation test analysis revealed that there was direct correlation among numbers of CD3 expressing cells and Iba1 and CD204 immunolabelled cells. Conversely, the expression of MUM1 was inversely correlated with CD163 immunolabeling (**Table 1**).

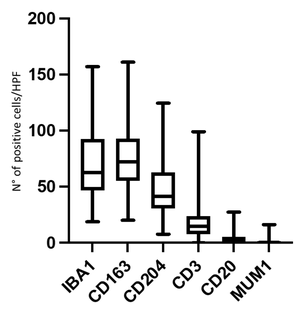


Figure 3. Box plot. CD20⁺ and MUM1⁺, cells were extremely underrepresented when compared to the macrophage populations (Iba1, CD163, CD204).

Marker		MUM1	CD163	CD204	CD3	CD20
IBA1	Correlation coefficient	0.026	0.120	0.379*	0.596**	-0.087
	Sig. (2-tailed)	0.888	0.507	0.027	0.000	0.636
MUM1	Correlation coefficient		-0.417*	-0.101	0.053	0.179
	Sig. (2-tailed)		0.020	0.583	0.775	0.327
CD163	Correlation coefficient			0.389*	0.221	0.023
	Sig. (2-tailed)			0.028	0.232	0.902
CD204	Correlation coefficient				0.451**	0.177
	Sig. (2-tailed)				0.009	0.340
CD3	Correlation coefficient					0.189
	Sig. (2-tailed)					0.310

Table 1. Spearman's Correlation analysis (Spearman rank correlation coefficient, ρ) among the analyzed immune populations. * significance p<0.05; ** significance p<0.01.

CD163 showed a frequent co-expression with Iba1, but the two cellular populations did not completely overlap (**Fig. 4A**). Differently, CD3 did not seem to co-localize with Iba1 (**Fig. 4B).**

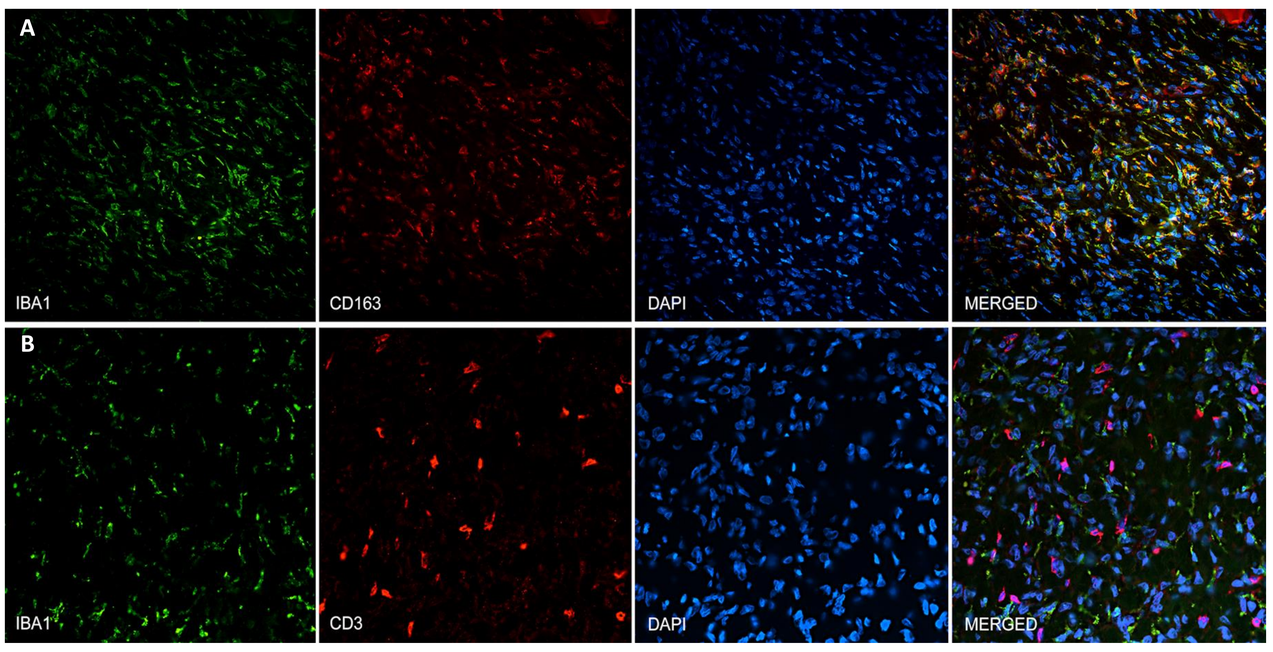


Figure 4. Double immunofluorescence. (A) Equine sarcoid. Iba1-CD163 colocalization. (B) Equine sarcoid. Iba1-CD3 colocalization. Nuclear counterstain was performed with DAPI.

DISCUSSION & CONCLUSIONS

Our findings suggest that the TIME in equine sarcoids is mostly characterized by a prominent macrophagic infiltrate, without conspicuous lymphocytes and plasma cells infiltrating the neoplastic tissue. The direct correlation between CD3 expressing cells, Iba1⁺, and CD204⁺ cells, together with the inverse correlation among MUM1⁺ cells and CD163⁺ cells, might suggest a potential role of CD163⁺ cells in driving immune response, as macrophages with M2 polarization. Additionally, their increase is not associated with an increase in TILs, which are commonly considered a valid index to evaluate the degree of immune response to the neoplasm. Our data, considered as a whole, seem to indicate that sarcoids may be considered "cold" tumors, characterized by macrophagic infiltrates and the absence of cytotoxic CD8⁺ TILs and other pro-inflammatory cells, such as B lymphocytes and plasma cells [3]. However, the data obtained require further validation on a complete follow-up series, that could help defining the biological and prognostic role of both the infiltration pattern (TILs) and the immune populations examined.

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