QUESITO 1 – Elenco domande

1. Immagina di dover validare un anticorpo specifico per un antigene di specie umana nella specie canina. Quale la procedura che seguiresti? Perché?

2. Durante il processo di esecuzione della metodica immunoistochimica, quali sono i pattern di reattività attesi per i seguenti marker?

MARKER	REATTIVITA'
c-Kit	
Ki67	
CD3	
CD20	
Iba1	
MUM1	
Papillomavirus	
Leishmania	
Canine Adenovirus type 1	
FIP virus	

3. Come organizzeresti l'armadio in sala settoria per una buona pianificazione delle attività lavorative, nel rispetto delle norme igieniche e di sicurezza nel luogo di lavoro?

4. Come organizzeresti e archivieresti in modalità digitale i dati anatomoistopatologici e laboratoristici all'interno di un ospedale veterinario?

5. [....] Confirmation of the Diagnosis of Acute Myeloid Leukemia

For all cases, flow cytometric analysis, using a panel of antibodies to lymphoid and myeloid markers (Supplemental Table S2), was performed on lymph node or bone marrow aspirates or peripheral blood at the time of referral or upon receipt of samples for the AML research study. In venous blood and bone marrow samples, we restricted our analysis to suspect tumor cells, ie, those with low to high forward scatter (FSC, size) and lower side scatter (complexity) than neutrophils. For CD34, positive ($^+$) labeling were defined as ≥5% of gated suspect tumor cells, whereas ≥20% labeled cells was used for all other markers.[31] For lymph node aspirates, cells with low FSC and intermediate to high FSC were gated separately, when possible, reasoning that residual non-neoplastic lymphocytes may fall into the low FSC gate. Similar to the cytologic assessment of the nodes, ≥5% labeled cells was used to indicate an infiltrate of either CD34$^+$ precursor cells or myeloid cells expressing one or more myeloid marker, including CD11b, CD11c, CD14, CD18, and CD4.[15,31] We included CD4 as a myeloid marker as it is expressed by mature canine neutrophils as well as T lymphocytes. We have previously used CD4$^+$labeling in cells that are negative ($^-$) for the T-cell markers, CD3 or CD5 or both, to identify AML.[31] Given that CD34$^+$ cells in an acute leukemia are usually major histocompatibility complex II (MHCII)$^-$,[15,31,32] whereas CD34$^+$ cells in B-cell lymphoma are MHCII$^+$,[37] we subdivided CD34$^+$ cells into CD34$^+$/MHCII$^+$ and CD34$^+$/MHCII$^-$subsets when possible. In addition, we quantified the percentage of CD14$^+$monocytes that were MHCII$^-$ when cells were co-labeled with both markers. Monocytes are normally MHCII$^+$ but can be MHCII$^-$ in acute leukemia.[15] Flow cytometric analysis with a similar panel of antibodies was done by other laboratories for the prospectively enrolled cases in the AML study (Supplemental Figure S1). [from Vet. Pathol. Acute myeloid leukemia with peripheral lymph node involvement in dogs: A retrospective study of 23 cases. Nov. 14, 2024]

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QUESITO 2 – Elenco domande

1. Descrivi la tecnica del paraffin block tissue-microarray. Quale applicazione intravedi per questa tecnica in oncologia veterinaria e quali vantaggi.

2. Quali sono le tecniche di allestimento e di processazione di un campione citologico a partire da:

   a) Versamento addominale
   b) Tampone auricolare
   c) Tessuto fresco non fissato
   d) Liquido cefalorachidiano
   e) Lavaggio bronchioloalveolare (BAL)

3. Nel caso in cui ci si trovasse ad analizzare visceri di mattatoio o cadaveri con lesioni compatibili con tubercolosi, quali procedure e dispositivi di sicurezza ritieni debbano essere messi in campo da parte dell'operatore, e nei confronti dell'ambiente e delle strutture di lavoro?

4. Ammettiamo che una ricerca raccolga una mole considerevole di dati da analizzare e sottoporre a indagine statistica. Quale/i software utilizzeresti e con quali funzioni?

5. Immunohistochemistry

[....] For the positive control, a section from a tissue (bovine and porcine lymph node and lung) known to express the protein of interest was included; for the negative and isotype controls, the primary antibody was replaced by the corresponding blocking solution or the isotype-match antibody of irrelevant specificity, respectively, to confirm the lack of non-specific binding. The corresponding biotinylated secondary antibody was applied for 30 min followed by Avidin-Biotin-Peroxidase Complex (Vector Laboratories) for 1 hour at room temperature in the dark. Labeling was visualized by NovaRED substrate kit (Vector Laboratories).

Digital Analysis

Immunolabeled slides were subjected to objective digital image analysis to determine the positive percentage (%) of CD172a+, calprotectin+, iNOS+, CD68+, CD107a+, Arg1+, and CD163+ immunolabeled cells. The slides were photographed at 100× magnification by digital and light microscopy (Olympus BX43, L'Hospitalet de Llobregat, Barcelona, Spain). Immunolabeled cells were registered for the different stages (I, II, III and IV) of tuberculous granulomas for each slide. All granulomas present in each slide were included in the study. The slides were digitalized and the whole area of the granuloma was selected as the region of interest (ROI), with the area of immunolabeled cells inside the ROI was calculated using QuPath software version 0.3.0[1] after setting thresholds for each marker and stage of granuloma. For stage III and stage IV granulomas, necrotic or mineralized centers were not included in the analysis, as previously described.[39] The results were expressed as the percentage of the area covered by immunolabeled cells within the whole area of the granuloma.

[from Vet. Pathol. Macrophage polarization in lymph node granulomas from cattle and pigs naturally infected with *Mycobacterium tuberculosis* complex
Vol.61, Issue 24]

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QUESITO 3 – Elenco domande

1. Quali sono i fattori che possono condizionare in laboratorio i risultati delle indagini di immunoistochimica eseguite in assenza di processatori automatici?

2. Quali sono i dispositivi e le accortezze da prendere in considerazione in sala settoria nel caso in cui si dovessero effettuare dei campionamenti per esami batteriologici?

3. Quali colorazioni speciali sono indicate in caso di:

	COLORAZIONE SPECIALE
Steatosi	
Accumulo di rame	
Fibrosi	
Cryptococcus neoformans	
Aspergillus fumigatus	
Actinobacillus lignieresi	
Lawsonia intracellulare	
Accumulo di polisaccaridi	
Accumulo di ferro	
Accumulo di amiloide	
Accumulo di calcio	

4. Quali strumenti di Office automation utilizzeresti per la condivisione dei dati tra ricercatori e per garantire un accesso alle informazioni autonomo, rapido e sicuro?

5. [...] In Vitro Assay of PDGF-A Effects on AOFB-01 Cells

AOFB-01 cells were cultured at 1×10^3 cells in 24-well plates at 37°C in a humidified incubator at 5% CO_2 and were maintained in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (Gibco) with 10% fetal bovine serum (Cytiva) and 1% antibiotics (penicillin-streptomycin, Fujifilm). The wells were separated into 2 groups. One group was treated with human PDGF-A (Gibco) solubilized in distilled water, and the final concentration of PDGF-A in medium was 20 ng/mL. Only distilled water equal to the volume of PDGF-A solution was added to the other group. Media were exchanged every 2 days to add fresh growth factor. The number of cells was counted manually using a hemocytometer at 2, 4, 6, 8, 10, 12, and 14 days after the initiation of cell cultures. This experiment was conducted in triplicate. A growth curve was constructed, and the cell population doubling time in the exponential growth phase was calculated using the least squares method. The average cell-doubling time with or without PDGF-A was examined using Student's *t*-test. The significance of differences was set at $P < .05$. Data are presented as the mean ± standard deviation (SD). AOFB-01 cells were also cultured at 100 cells per well in 96-well plates as described above and separated into 2 groups with 20 ng/mL PDGF-A or distilled water only. Each group consisted of 9 wells. Media were exchanged every 2 days to add fresh growth factor. On day 8, the CCK-8 solution (Cell Counting Kit-8, DOJINDO) was added to each well and allowed to stand for 2 hours to color. Absorbance was measured using an iMark microplate reader (Bio-Rad Laboratories). Negative control wells were prepared without tumor cells. Average absorbance with or without PDGF-A was tested using Student's *t*-test. The significance of differences was set at $P < .05$. Data are shown as the mean ± SD. [from Vet Pathol. The relationships of platelet-derived growth factor, microvascular proliferation, and tumor cell proliferation in canine high-grade oligodendrogliomas: Immunohistochemistry of 45 tumors and an AFOB-01 xenograft mouse model. Vol.61, ISSUE 5]

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QUESITO 4 – Elenco domande

1. Quali accortezze e strumentazioni sono da considerare nell'ipotesi di asportare il sistema nervoso centrale da un cadavere in sala settoria?

2. In caso di campione citologico prelevato da sospetto mastocitoma scarsamente differenziato, quale colorazione citologica prenderesti in considerazione?

3. Durante il processo di esecuzione della metodica immunoistochimica, quali tessuti controllo scegli per i seguenti marker?

MARKER	REATTIVITA'
c-Kit	
Ki67	
CD3	
Citocheratina	
PNL2	
SOX10	
Calponina	
CD31	
Sinaptofisina	
vimentina	
GFAP	

4. Quali software utilizzeresti per gestire riunioni a distanza, e quali gli strumenti per condividere dati e immagini su cui lavorare in live?

5. Cell Line and CoCl2 Treatment

[...]

Immunofluorescence

To investigate the effects of $CoCl_2$ on HIF1-α nuclear translocation and CAIX expression, immunofluorescence against HIF1-α and CAIX was performed on OLGA cells exposed to the highest $CoCl_2$ concentration (200 μM) for 12, 24, and 48 hours. Briefly, 2×10^4 cells were plated in 8-well chamber slides (Lab-Tek II Chamber Slide System; Nalge Nunc International Corporation) until 70% confluence. After treatment, cells were fixed with methanol:acetone (1:1 proportion) for 30 seconds. After washing 3 times with Tris-HCl (0.1 M, pH 7.6), cells were blocked with 10% normal goat serum for 1 hour at room temperature and then incubated overnight at 4°C with antibodies to HIF-1α (1:100) and CAIX (1:200). After washing with Tris-HCl, cells were incubated with a fluorescent secondary Alexa488-conjugated goat anti-rabbit IgG antibody (1:500 dilution, ThermoFisher) and anti-mouse IgG (1:500) for 1 hour in the dark. Subsequently, cell nuclei were stained with DAPI (0.5 μg/ml in Tris-HCl, Sigma-Aldrich, USA) for 10 minutes, washed 3 times with Tris-HCl, and then the sections were covered with mounting medium (PermaFluor, Thermo Scientific) and kept overnight in the dark. Fields were randomly selected by microscope TCS SP8 (Leica Microsystems CMS GmbH Mannheim, Germany) and z-stacks of 10 nm were acquired. Z-stacks were then processed directly with the Leica LAS-X software to produce maximum projection images of each field. To detect colocalization of different fluorochromes, fluorescent signals in different channels were merged to produce multicolor images. Image acquisitions were performed with a resolution of 1024 × 1024 pixels with a 200 Hz sampling frequency. [From Vet Pathol. Hypoxia-associated markers in the prognosis of oral canine melanoma. Vol.61, Issue 5]

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