Paws claws and udder things

Gribbles VETERINARY

July 2021

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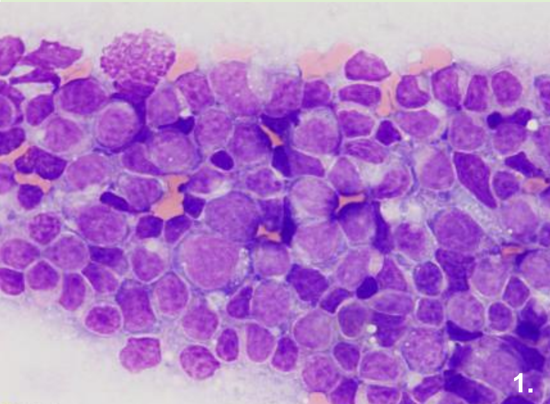

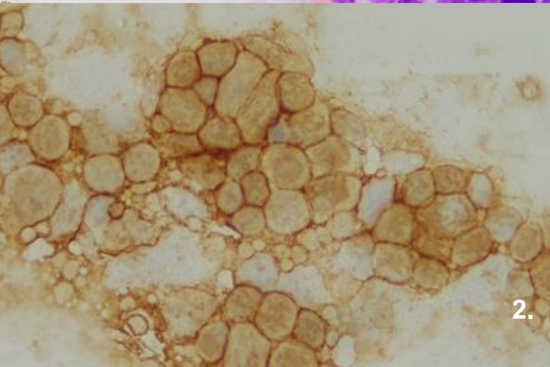

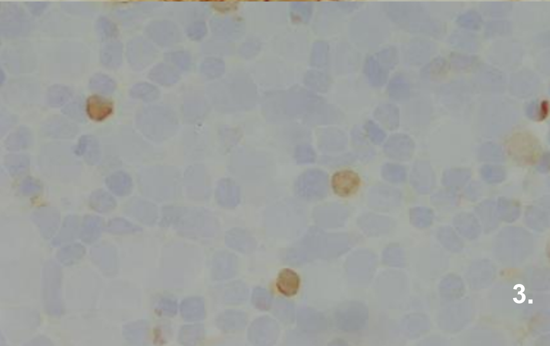

Dogs, lymphoma and ICC

SUNAO FUJITA

Immunocytochemistry (ICC) is an adjunct diagnostic tool, which helps to identify cellular origin utilizing the accuracy of antibody-antigen binding. In comparison with immunohistochemistry (IHC), which is more commonly used in both human and veterinary medicine, ICC is not routinely exploited for the classification of a variety of neoplastic lesions.

However, ICC has the potential to be a valuable diagnostic tool, not only to identify cellular origin, but also to obtain prognostic information about neoplastic diseases. As of now, ICC is primarily used for immunophenotyping of lymphoid neoplasms (lymphoma and lymphocytic leukaemia) in veterinary medicine, especially classification of B- or T-cell neoplasms. While IHC remains the gold standard for immunophenotyping of lymphoid neoplasia, excellent correlation between ICC and IHC has been reported in publications[1]. Gribbles offers two lymphoid cell markers for samples from dogs, CD20 for B-cells and CD3 for T-cells.

The majority of lymphomas in dogs are not challenging to diagnose on routine cytology samples, particularly on aspirates of peripheral lymph nodes. However, immunophenotype and evaluation of prognosis cannot be determined from the cytology alone. Immunophenotyping is one of the crucial elements for classification of canine lymphoma. Treatment plans (chemotherapy protocol) and prognosis can vary depending on the ICC results. Sometimes, round cell neoplasm might be difficult to distinguish by morphology alone. Utilizing the lymphoid cell markers, CD3 and CD20, can be beneficial for confirmation of lymphoid origin.

Figure 1. Wright's-Giemsa stain—canine lymphoma; Figure 2. CD20 stain—the majority of lymphocytes show positivity, primarily intermediate-large in size; Figure 3. CD3 stain—a few cells show positivity, small lymphocytes.

ICC is used to immunophenotype lymphoid neoplasms.

Basically, ICC is not an option to confirm the diagnosis of lymphoma in suspect or nebulous cases. It is NOT a test to differentiate lymphoma from a normal or hyperplastic lymphoid population on lymph node cytology samples. ICC is only an adjunct test to provide immunophenotype information.

Furthermore, definitive subtyping requires evaluation of tissue architecture by means of histopathology in addition to immunophenotyping (e.g. indolent lymphoma).

Advantages of ICC

- Minimally invasive and low cost of sampling procedure – use of FNA sample
- Low anaesthetic risk
- Easy to re-sample if non-diagnostic results obtained
- Can quickly provide pre-surgical diagnosis in cases requiring surgical treatment.

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Limitations of ICC

- > Significant variation in cytology slide quality because of using FNA slides
- > Multiple identical slides are not available
- > Not helpful to confirm a neoplastic proliferation

Samples

At Gribbles Veterinary, the samples we usually receive for ICC are direct smears of FNAs taken from enlarged lymph nodes . FNA samples are very easy to obtain, but their sample quality varies quite a bit depending on sampling technique.

Lymphocytes are inherently fragile and are easily ruptured, resulting in lack of adequate numbers of intact cells and abundant cytoplasmic fragments in a background of smears. Additionally, inadequate cellularity can also be a problem (sometimes, no lymphoid cells are harvested). These issues can cause difficulties with interpretation of ICC slides.

Therefore, we perform routine staining (Wright Giemsa stain) on the slides and confirm whether these smears have good quality enough for ICC before processing ICC. Alternatively, we can use pre-stained slides submitted for routine cytologic examination for subsequent ICC, if they are already diagnosed with lymphoma by a pathologist.

While generally unstained slides yield stronger immunocytochemical responses, the difference of the signal intensity is minimal between unstained and Romanowsky-stained (e.g. Wright-Giemsa, Diff Quik) slides. Furthermore, the difference of signal intensity does not affect the diagnosis of immunophenotyping[2].

Air-dried cytology slides are acceptable for use in ICC as described above, but cell viability and antigen-antibody responses are decreased if the slides remain at room temperature for a long time. If you want to keep air-dried slides for use of ICC in your clinic, storage at 2°C to 8°C for up to 2 weeks before ICC does not appear to affect antigenicity. The slides should be put in a plastic slide box and then in a zip-lock plastic bag with desiccant. Samples need to equilibrate to room temperature before opening the bag to avoid cell rupture[3].

Lymphoid cell phenotype can also be determined via flow cytometry (FC) and PCR for antigen receptor rearrangement (PARR). FC and PARR are also minimally invasive tests, but they are not routinely available domestically at present. Note that we are able to send slides overseas for PARR testing; however, turnaround time can be prohibitive in some cases. Therefore, ICC is a routinely available test without extra time and effort to the submitting veterinarian to identify the immunophenotype of lymphoid cells in dogs.

References:

1. Sapierzynski, R. Practical aspects of immunocytochemistry in canine lymphomas. *Polish Journal of Veterinary Sciences*, 13: 661-668, 2010
2. Raskin, R. E., Vickers, J., Ward, J. G. et al. Optimized immunocytochemistry using leukocyte and tissue markers on Romanowsky-stained slides from dogs and cats. *Veterinary Clinical Pathology*, 48: 88-97, 2019
3. Raskin, R. E., Meyer, D. J. Advanced Diagnostic Techniques. In: *Canine and Feline Cytology*, 3rd ed. (Wilson, L. ed.), pp. 453-494, Elsevier, St. Louis, 2016.

Thanks for participating!

In our May newsletter we gave you the option of participating in a very short survey to help us better understand how you prefer to receive our news and updates.

Thank you to everyone who took part and provided feedback!

This feedback will help us tailor our content and the way the information is delivered to you, ensuring we reach as many of our clients as possible.

The good news is nearly 60% of respondents read our newsletter most months and 90% accessed it either via the links in the email or the attached PDF. The posts on our Facebook page were preferred by just over 30% of people.

Clearly we're hitting the mark with our *Case of the Month*, as interesting cases are by far the most popular article in each issue. We also notice this on our Facebook page with our *What's up doc?* segment, *Down the scope*, and *Pop-quizzes* being big drawcards. Sample handling and packaging, laboratory tips and tricks seem to be other prized article types.

Did you know, all the articles from our newsletters can be found in the NEWS section of our website? They will all also feature on our Facebook page during the month. Sign up to receive updates first-hand here.

Liver sampling

ROB FAIRLEY

When sampling the liver for histology (especially for cases with known liver disease) it is helpful to take multiple pieces and it is really helpful to make sure that what you select includes some decent-sized bile ductules. Facial eczema, for example, preferentially affects the larger ductules.

Some livers may have really badly affected areas (the end of the left lobe in facial eczema is a classic). You can sample the badly affected area but make sure you sample less affected areas as well, where the changes are often more diagnostic.

Case of the month

KATHRYN JENKINS

Fine needle aspiration (FNA) can quickly and easily identify whether an enlarged lymph node is hyperplastic/ reactive, inflamed, or neoplastic (e.g. lymphoma). It can also aid in the identification of potential metastatic disease (e.g. mast cell tumours, carcinoma, histiocytic sarcoma), and can also identify uncommon infectious agents.

A recent case highlighted the usefulness of lymph node FNA when identifying the cause of a skin nodule in a young cat.

Clinical history:

A one-year-old female spayed domestic shorthair cat was presented for a 6mm mass on her lower lip, and significant right submandibular lymph node swelling. The mass had appeared quickly, but there had been no change in size over the previous 10 days. FNA was performed on the lymph node, and the mass was biopsied and sent for histopathology.

Laboratory findings:

Cytology of the lymph node contained a well -preserved population of mixed lymphocytes, with scattered large epithelioid macrophages, and low numbers of neutrophils, amidst scant amounts of blood on a basophilic proteinaceous background (Figures 1 and 2).

The lymphoid population were mostly small mature lymphocytes, with fewer intermediate lymphocytes and rare large lymphocytes, with occasional plasma cells and reactive appearing lymphocytes. Epithelioid macrophages had moderate amounts of pale basophilic cytoplasm, oval to slightly irregular nuclei, with stippled chromatin and 1-2 small nucleoli. The cytoplasm contained small numbers of negatively staining thin refractile rod-shaped structures, consistent with Mycobacteria. One of the cytology smears was submitted for additional acid-fast stains, which was positive, providing further confirmation of Mycobacterial infection.

Histopathology of the lip mass comprised a dense nodular multifocal to coalescing infiltrate of macrophages and neutrophils, with multifocal areas of necrosis and haemorrhage. This pyogranulomatous inflammation was supportive of a diagnosis of Mycobacteria, however these organisms are not readily observed on routine H&E sections. Subsequent acid-fast stains were positive for small numbers of Mycobacterial organisms, confirming the diagnosis of cutaneous Mycobacteriosis, with dissemination to the draining lymph node.

Discussion:

Classical feline leprosy syndrome, can be caused by several slow growing and fastidious species (including *Mycobacterium lepraemurium, M. visibile, M. tarwinense*, and *M. leparefelis*). The syndrome is progressive, starting with cutaneous nodules, with possible spread to regional lymph nodes later in the course of disease, followed by dissemination to internal organs (such as liver or spleen). Occasionally they can demonstrate a more aggressive clinical course. Excision of solitary lesions can prove curative, however adjunctive antimicrobial therapy (using two or three drugs effective against slowly growing mycobacteria) is recommended, especially once disease progression has been identified.

The organisms involved with classical feline leprosy syndrome are not thought to pose a zoonotic risk. However, this contrasts with tuberculous forms of Mycobacteria (such as *M. bovis*), which do have zoonotic potential. *M. bovis* has previously been isolated from domestic cats throughout New Zealand, and historically these cases arose from areas where *M. bovis* was also present in the wildlife population (especially the brush-tailed possum). Lesion distribution in these cats included the skin, submandibular and mesenteric lymph nodes, as well as generalised infection. Recent cases in the United Kingdom have also been linked to the feeding of raw pet food diets.

Given Mycobacteria species cannot be differentiated by cytology or histopathology alone, confirmation of Mycobacteria species is recommended. Mycobacteria are often non -culturable, and speciation of Mycobacteria can be achieved by PCR on fresh tissue, which is the preferred sample type. Tissue from cytology slides and formalin fixed tissue may also be utilised, in cases where fresh tissue is not available.

PCR was performed on both fixed and fresh tissue submitted for the case, with the PCR product from fresh tissue being 100%

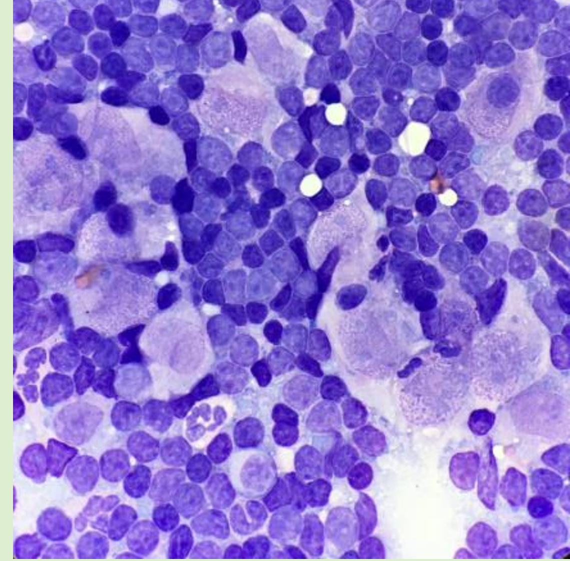
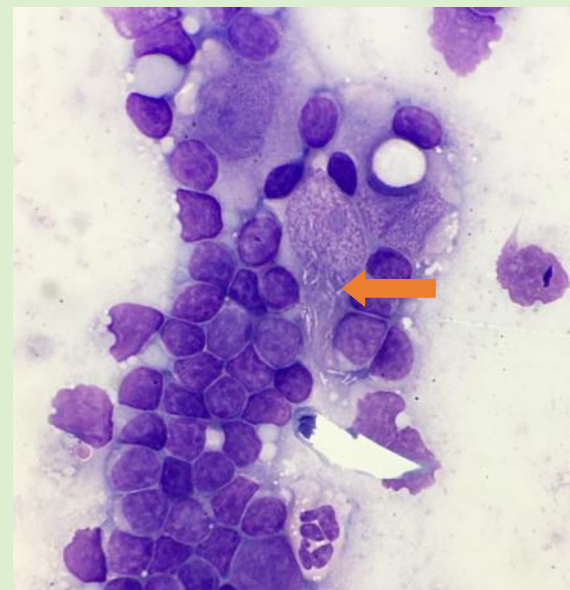


Figure 1. Cytology from the right submandibular lymph node (50x oil).

Figure 2. Macrophages containing negatively staining thin refractile rod-shaped structures (100x oil).



identical to *M. lepraemurium*. This is the most common cause of classical feline leprosy syndrome, with lesions commonly found on the head area in young cats. Low bacterial numbers in both cytology and histopathology are not uncommon, although high bacterial numbers can also occur. These lesions often ulcerate later in the course of disease, with dissemination uncommonly reported.

Thank you to Brendon Bullen from Kelburn Vets for submitting this interesting case.

References:

O'Brien, C. et al. Feline leprosy due to Mycobacterium lepraemurium. Journal *Feline Medicine and Surgery*, Vol. 19 2017.

O'Halloran, C et al. Tuberculosis due to Mycobacterium bovis in pet cats associated with feeding a commercial raw food diet. *Journal Feline Medicine and Surgery*, Vol. 21(8) 2019.

De Lise, G.W. et al. A report of tuberculosis in cats in New Zealand, and the examination of strains of Mycobacterium bovis by DNA restriction endonuclease analysis. *New Zealand Veterinary Journal*, 39, 1990.

Consumable of the month

Do you order laboratory consumable items from us online or via our order form? If you need just one blood tube or swab, or enough for a herd, we've got you covered.

Our featured consumable item in July is **faecal egg counts (FEC) kits**.

These kits contain everything you require for collecting samples for multiple FECs. They are perfect for passing on to farmers or using yourself when out in the field. They come packed with 10 samples pots, disposable gloves, a custom request form, a 'How To' guide that runs through the whys and hows for collecting the samples, a cardboard box to stow it all in, and your choice of with- or without a courier bag to send it to us.

Couldn't be easier.

2021 Price book errata

Since sending our new price book out via email, we have picked up a few errors. Here are some corrections:

- Neospora IFAT testing is $36.27
- Leptospira PCR testing is $61.37
- Toxoplasma antibody testing is $26.50

All pricing above is excluding GST and applies to all animal types.

Can't log in to place an order?

If you're after some consumables, but can't log in to our online shop to place the order, here are the most common reasons why:

1. You haven't set up a user account with us yet (this is different to your eResults account).
2. You can't remember your username ⏩ most times this is the word before the @ in your email address.
3. You can't remember your password ⏩ hit the LOST YOUR PASSWORD? button to reset (this can be done with username or email address).

If you get locked out, go take a break and try to reset again in about an hour. If all else fails, give us a call and we'll help you get sorted!

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