HGVT

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PARAFFINALIA NEWSLETTER

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March 2024

The HGVT aims to provide a dynamic continuing education program in which all persons with an interest in Histology and Histotechnology are freely invited to participate.

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ABN 49725 623 468

Committee Page

The members of the Histology Group of Victoria 2024 are:

Name	Institution
Samantha Arandelovic	Mater Hospital Brisbane
Kerrie Scott-Dowell	Dorevitch Pathology/ Leica Biosystems
Mark Bromley	Sullivan Nicolaides Pathology
Kellie Vukovic	Melbourne Pathology
Alistair Townsend	Royal Hobart Hospital
Christine Gorringe	Royal Hobart Hospital
Elizabeth Baranyai	Cabrini Health
Bronwyn Christiansen	Royal Children's Hospital
Tu Anh Huynh	Royal Melbourne Hospital
Snejana Ursache	Alfred Hospital
Gulnur Orman	Box Hill Hospital
Dodie Pouniotis	RMIT University
Fatema Tajbhai	Northern Health
Kerrie Howard	Northern Health/ RMIT University
Li Shan Ong	Monash Pathology/ Melbourne Pathology
Enia Kakaflikas	Pathology Solutions
Maria Chavez	Monash Pathology

Contact us at
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General enquiries: secretary@hgv.org.au
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Presidents Address

Behind the Bench with Sam Arandelovic

I hope everyone had a lovely break over Christmas and I know that sounds like a long time ago. The weather has been unpredictable and I hope that everyone is safe and not affected by the storms or bushfires.

The committee has been working very hard to organise this year scientific meetings and we hope that the topics are of interest to you. Also, Kellie who is still on maternity leave is working very hard to find a venue for Trivia night in July that can fit more than 150 people. Keep the night of Friday 26th July free!

This year is packed with full of Histology goodness and a hope you are all looking forward to it and applying for professional development leave.

Firstly, there is the DIHC Conference in Tweed Heads 24th-26th of May with Digital Pathology component. Digital Pathology is definitely gaining a lot of traction in Histology and something to keep an eye on.

Then there is the National Histology Conference in August 9th- 11th in Sydney- Darling Harbour, after five Covid enforced years of absence.

Both programs are looking great and contain many things that will interest many people.

Looking forward to seeing you all at our next Scientist meeting in early May.

Hope you all have a lovely Easter!

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Under the Microscope

with Kerrie Howard

What was your first part time job?
I worked in a little Italian restaurant my family had frequented for years as a waitress and kitchen hand.

What is your current Job?
I have just started at Northen Health as the Cut-up Supervisor! I am very excited for this role and to grow as both a scientist and leader. Before this, I worked at Dorevitch Pathology for a long time, growing from a baby tech to a senior scientist.

How long have you been working in your role?
I have been at Northern Health for about a month. I have been on and off at RMIT for about 4 years.

What skill do you want to learn and why?
I would love to refine (re-learn... Let's be honest) my skills with playing the piano and guitar! I miss music and love how relaxing it can be to play.

If money was no object, what would you do all day?
I would work with animals! All over the world, volunteering to help in rescues and rehabilitation organizations. It would mean I could travel and be surrounded by animals, which would be perfect.

What's an ideal weekend for you?
An ideal weekend would be spent hiking or at the beach with loved ones and dogs. Anything to spend time outside.

What's on your bucket list this year?
This year I would love to be more intentional with my time and ensure I get the most out of all my free time- whether that be relaxing or travelling.

What music/podcast is on your playlist at the moment?
We would all be lying if we didn't say TSwift was taking up most of our playlists at the moment. A podcast I regularly listen to on repeat is "Just the Gist" that sells itself on giving you enough information about a topic to get you through a dinner party.

Where do you most want to travel, but have never been to?
I cannot wait to travel to Africa and South America.

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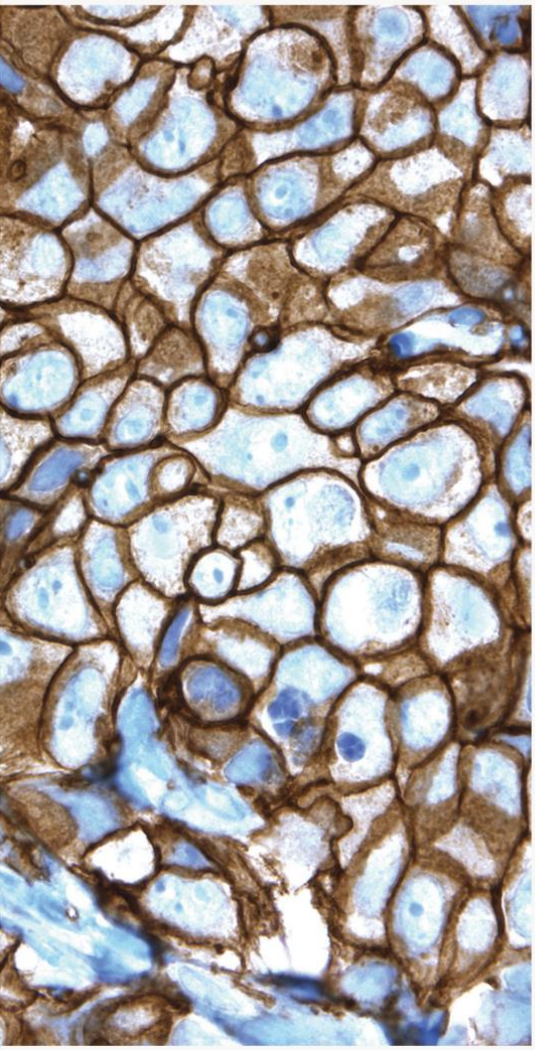

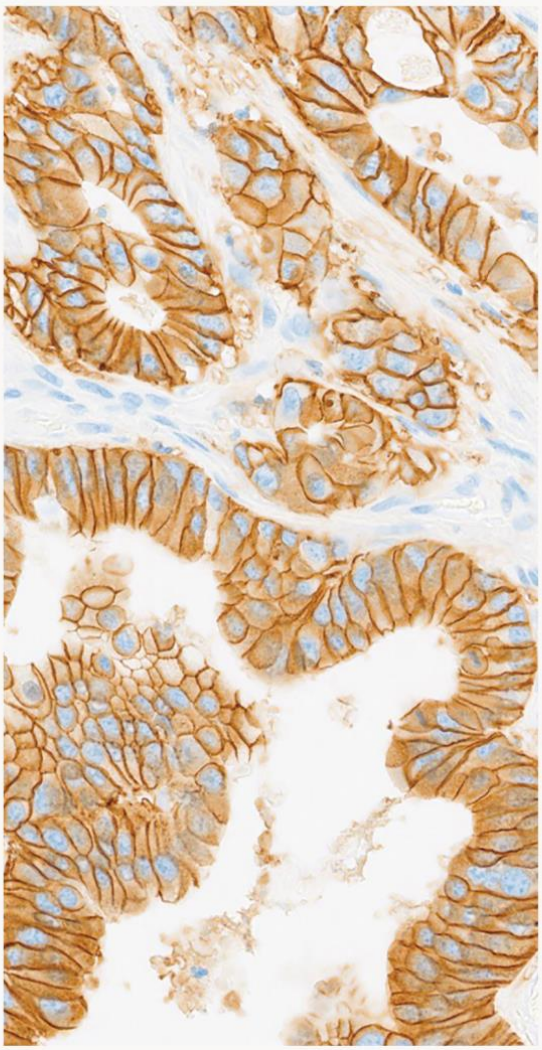

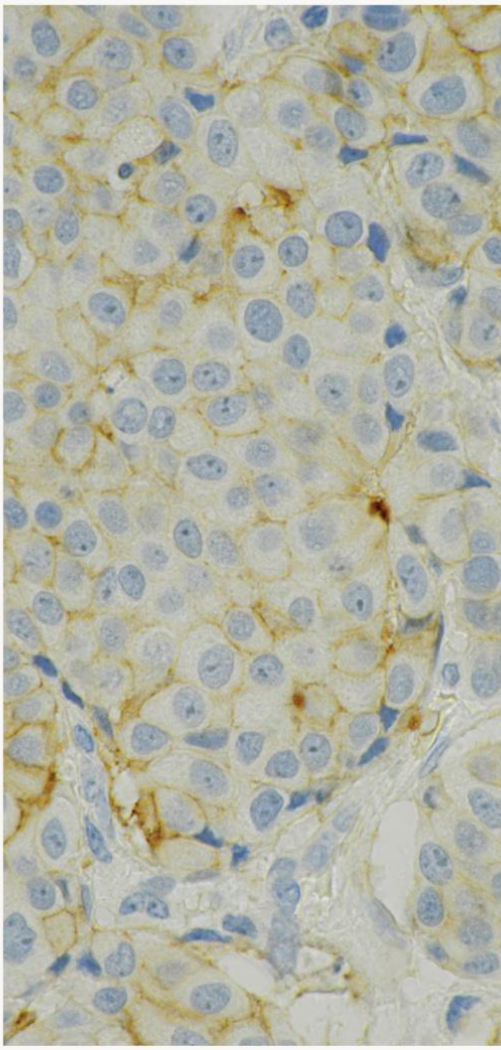

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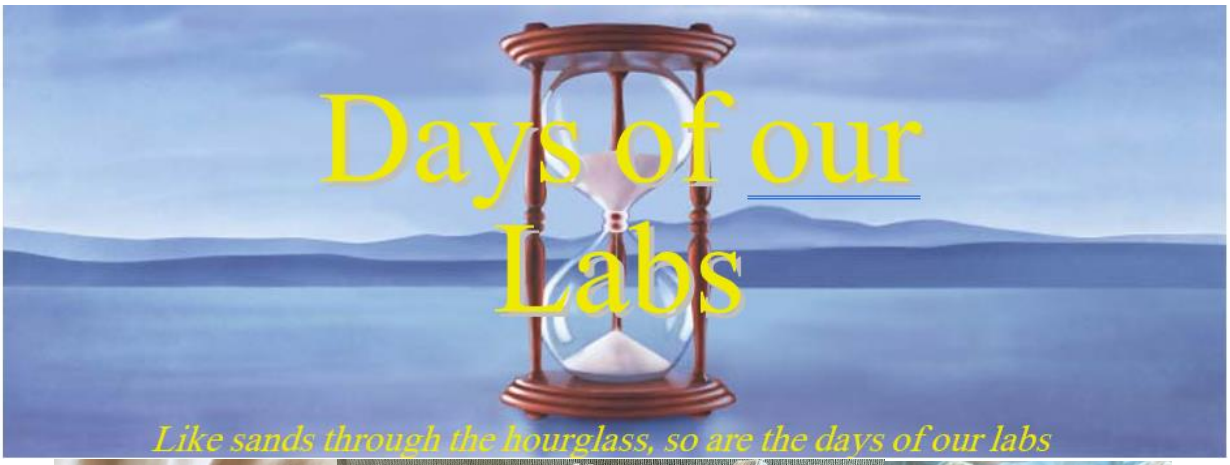

Congratulations!! to Kellie Vukovic from Melbourne Pathology on the safe arrival of Harrison Flynn Carroll born on 28th November 2023.

A huge congrats to AP Northern Health for their successful" Go Live" on the 5th of Feb!

Dorevitch Pathology celebrating their Christmas party with" Tacky Trivia" at Burnley Brewing

Who saw Tay Tay? Histology News, Births, Marriages, Retirements??? Any news!!! We would love to hear from you! Submit a pic and a short description to "Days of our labs" to the HGVT Facebook messenger or email editor@hgv.org.au

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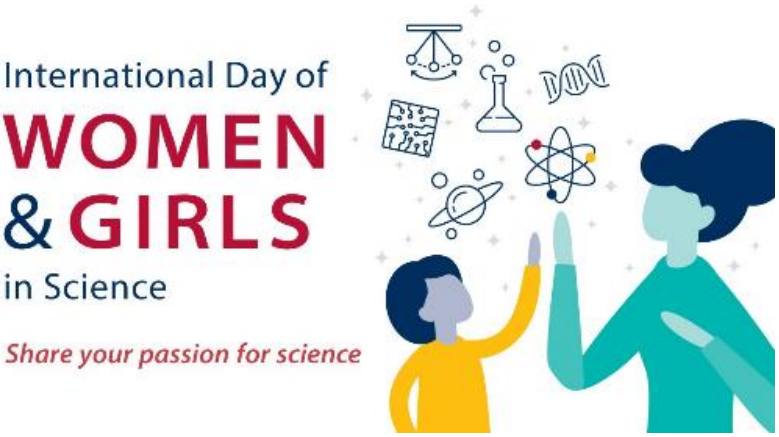
In the news

A look into Anatomical Pathology at RMH in the diagnosis of Lung Cancer
https://www.youtube.com/watch?v=mlseNaWoQ5c



11th of Feb was the International Day of Women and Girls in science – Shout out to all the women and girls in science!!!
A little piece of trivia: 82% of our HGVT committee is female

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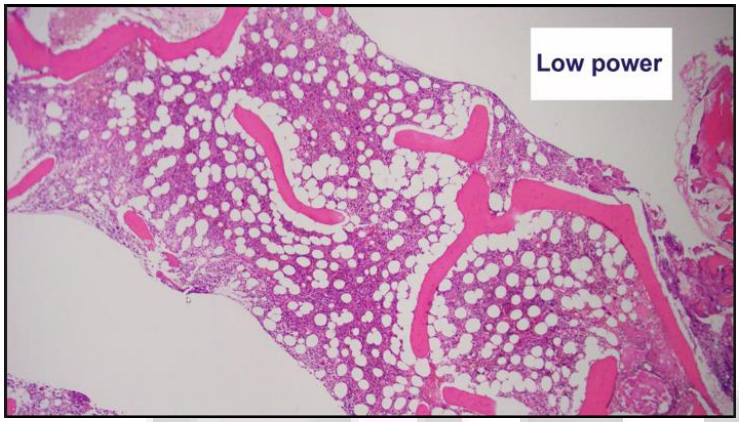
Review of Scientific Meeting

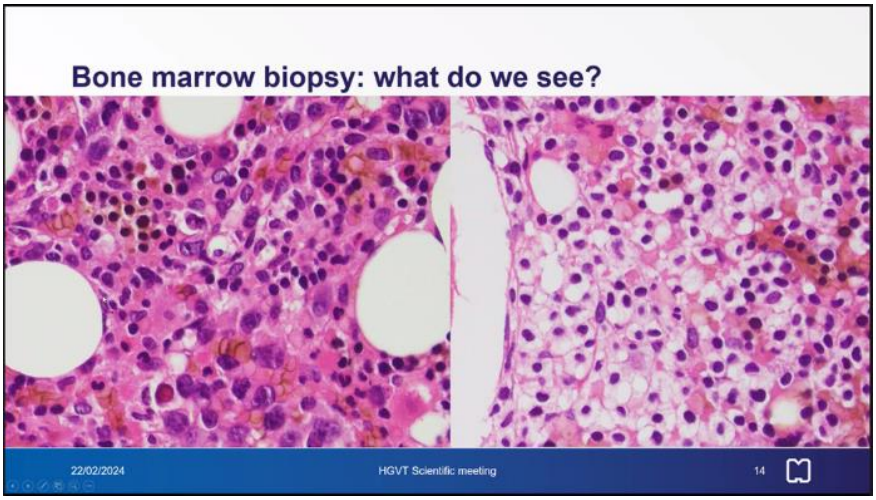
Bone Marrow Trephines

How different Lab's handle BMTs & diagnostic considerations including IHC
Zoom Meeting | 22nd February 2024 |Reviewed by: Kerrie Howard

Dr. Andrew Giang of Monash Health discussed the process of taking and analysis of bone marrow biopsies. Bone marrow biopsies are taken in two parts; A liquid aspirate which is taken for a slide smear (<0.5mL) and then collected in Lithium heparin and EDTA tubes for tests such as Flow Cytometry, cytogenetics, DNA/RNA extraction for molecular. Bone Marrow trephines and Aspirate are taken routinely from the Iliac spine with the use of a Jamshidi biopsy needle. The catalyst for this examination includes when there is requirement for analysis of haemopoietic tissue for diagnosis of Leukemia. However, they can be used for non-haemopoietic diseases such as granulomatous diseases such as Sarcoidosis, infections such as Tuberculosis or solid organ tumours.



The aspirate is taken to examine the quality and morphology of the cells in the bone marrow, whereby we can look at the tri-lineage hematopoiesis and other elements such as lymphocytes, plasma cells, histocytes. The thinner spread the aspirate is, the more ideal it is for examination. The Trephine sample complements this by also allowing examination of haemopoietic architecture at multiple levels to allow for an accurate, non-biased diagnosis to be achieved. The use of IHC techniques can also be completed on the trephine specimens such as CD68 for histocytes and macrophages, CD61 for megakaryocytes, CD3/CD20 for lymphoproliferative disorders or CD138 for plasma cell-based myelomas.



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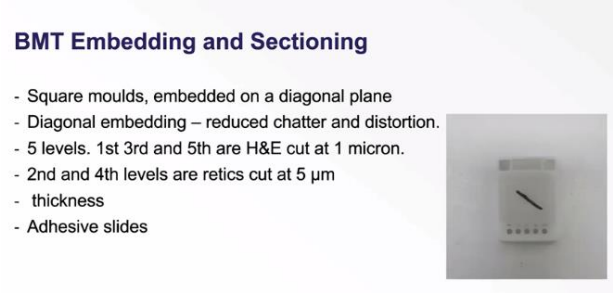
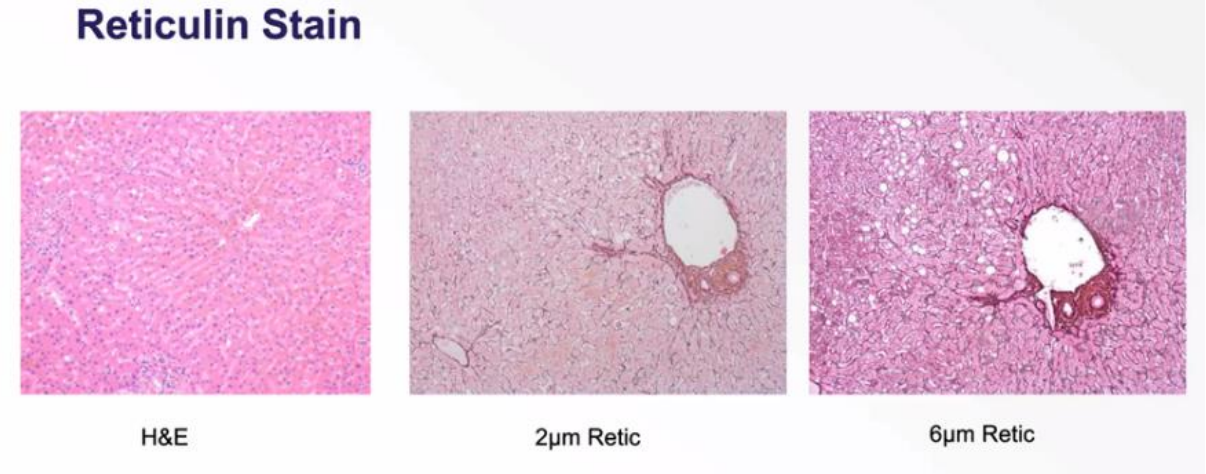
Upendra Chand, senior cutup scientist at Monash Health discussed the laboratory processing of bone marrow trephine specimens. He noted how the specimens were received in the lab within 1 hour of the specimen being taken from the patient. The fixation adequacy of the specimen is assessed with 3-4 hours of fixation being sufficient for most specimens. The specimen in then macro'd, washed in water for 5 minutes before being decalcified for 2-2.5hrs in 10% hydrochloric acid. HCL is an organic acid that removes calcium salts from the tissue to soften it, this can however damage DNA, RNA and proteins causing issues if specimen required molecular testing.



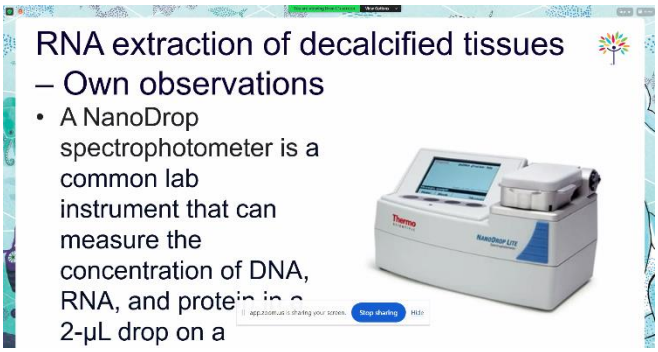
Once processed, the specimens are then embedded on a 45-degree angle and 5 sections are cut showing a H&E stain at 1 micron on L1,3,5 and a retic stain at 5 microns on L2,4. The levels are used to show a non-biased, more cohesive sampling of the specimens; especially with focal lesions that some leukemias and cancers commonly present as. Retic stains are used commonly throughout most laboratories which show the reticulin fibers within the connective tissue, the degree of this staining can assist greatly with diagnosis.



Bronwyn Christiansen; Principal scientist of the Royal Children's and Women's Hospital noted the difference between the strong (nitric acid, hydrochloric acid), weak (formic acid), chelating agents (neutral EDTA, i.e. Osteosoft) and the degree of issue it presents with DNA, RNA and protein analysis. Quality assurance considerations that Bronwyn suggested include timing decalcification, size the specimens, the type of fixation method and the length of fixation as well as the quality control procedures undertaken for in-house concoctions of declassification fluids.

Bron also analysed of whether matched controls were used (having the same time and decalcification conditions) and whether negative and positive controls we used that also underwent these conditions. She also noted how there is no current quality assurance program addressing immunohistochemistry on decalcified solutions, highlighting how their current reports suggesting that decalcification causes false negative even acoustic chemistry on surgical specimens and how the acknowledgement that decalcification was used on the specimen is critical to ensuring that there are no down flow negative effects. Furthermore, Bron acknowledged that

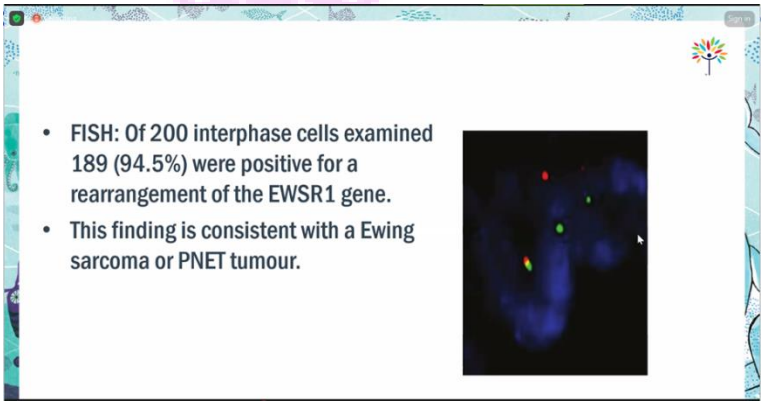
specimens that were bone marrow trephines versus those that were bone metastasis from other primary origins may need different declassification regimes especially if the specimens are requiring molecular studies.



The Royal Children's Hospital has use of the NanoDrop spectrophotometer which yields results of both the quality and quantity as well as the purity of DNA and RNA on a 260/280 nanometer ratio. A ratio of 1.8 is generally accepted for DNA and a ratio of 2 is generally accepted for RNA.

Bron gave a clinical example of a 10-year-old boy who presented to the emergency department with swelling of the leg post fall, an MRI imaging showed bone swelling and a bone biopsy was taken with a fresh imprint specimen sent for FISH testing as well as a specimen sent for Histology.

The slide sent for FISH testing was fixed in a methanol/acidic acid concoction and yielded results showing that 189 out of 200 examined cells were positive for a rearrangement. This is done through imaging showing fluorescent probes, the rearrangement showed 2 discrete colours instead of one joined colour that would be expected for the gene. This coincided with the histological diagnosis of Ewing sarcoma Which was processed through a 10% formic acid for 10 hours. Furthermore,



there was RNA extraction on the NanoDrop spectrophotometry which showed a yield of 1.89 purity for RNA, whilst not ideally pure the specimen was ran showing a genetic mutation of fusion of EWSR1- FLI1 gene, thus showing how Histology and molecular pathology of vital to confirmed diagnosis.

Thank you to all of our presenters on this interesting topic.

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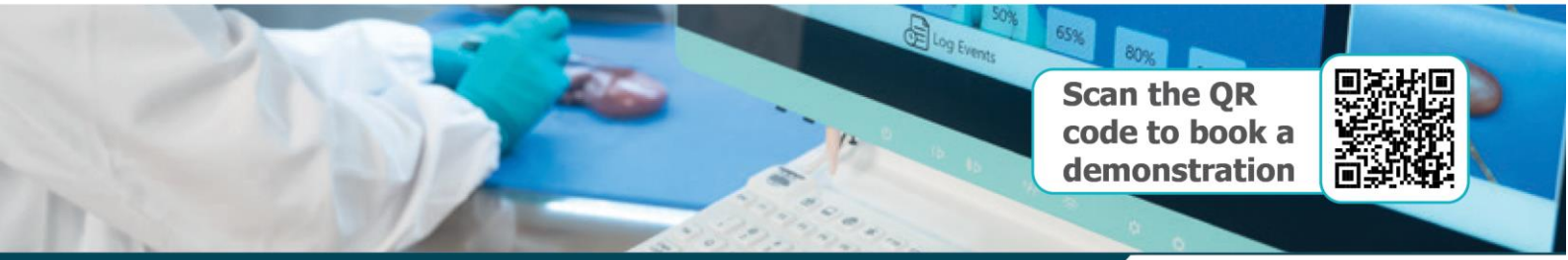

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Case study: Amyloid Associated Alopecia

Org. No A0035235F

By: Maria Boyer, *Monash Health Pathology*

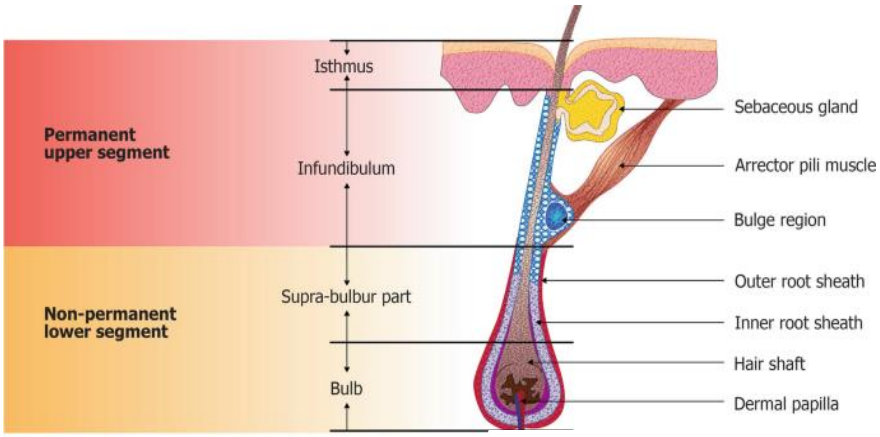


Fig 1. Histology of hair follicles

Amyloidosis is a group of complex diseases caused by the progressive accumulation of defective proteins in organs and tissue. At an ultrastructural level, amyloid proteins appear as non branching fibrils of indeterminate length with a diameter of 7.5 -10 nm and express the characteristic cross β pleated sheet conformation. Amyloidosis can lead to organ dysfunction and whilst it can affect any organ, amyloid associated alopecia is a rare finding. According to the literature, the alopecia is usually seen in patients with systemic amyloid disease and patients clinically present with a non scarring type of alopecia.

In all cases of alopecia adequate tissue sampling and appropriate laboratory processing is crucial for an accurate histopathological diagnosis, generally requiring two skin punch biopsy samples where one is sectioned vertically and the other horizontally to allow complete qualitative and quantitative assessment of the hair follicle from the bulb to the acroinfundibulum.

Case Study

65 year old female presented with 18months progressive severe alopecia.

Clinical Notes:

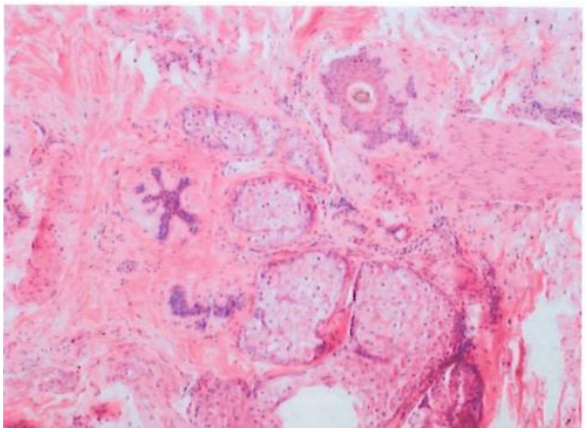
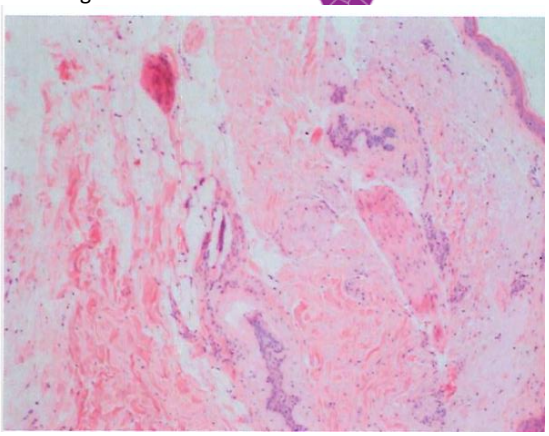
Biopsy x 2 scalp. 18 months progressive severe alopecia ? autoimmune,
? Female pattern hair loss,? Secondary to Heamatological disorder (monoclonal gammopathy of undetermined significance.

Macroscopic description:

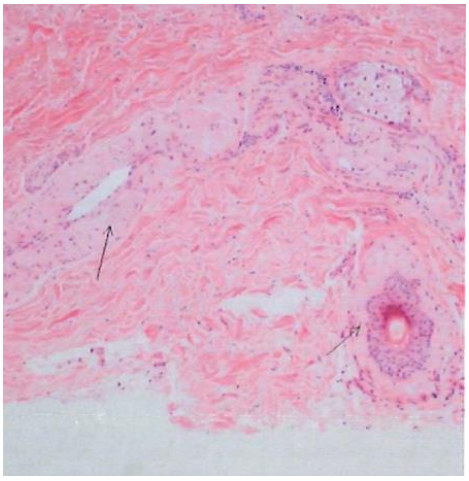
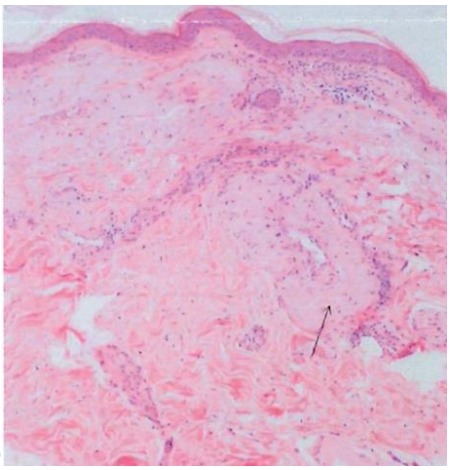
The specimen consists of two portions of tissue. The first portion is a 4 x 3mm skin punch (bisected horizontally) with a cream surface, all submitted. The second portion is 4 x 3 mm skin punch with a cream surface (bisected vertically) all submitted.

Microscopy:

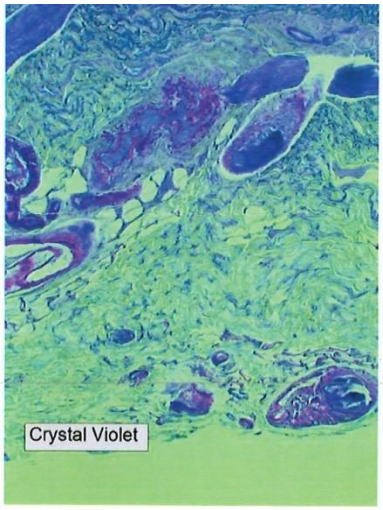
These punch biopsies from the scalp were examined in both horizontal and vertical planes. Both samples show a marked decrease in hair follicles with prominent miniaturisation as well as telogen shift, with all of the miniaturised follicles showing telogen morphology. No normal terminal anagen hairs are identified within the subcutis. On examination through the levels there is evidence of deposition of pale amorphous eosinophilic material within blood vessel walls as well as within the connective tissue sheaths surrounding the hair follicles. There is mild atrophy of the epidermal surface. No amyloid deposition is evident within the papillary dermis. A patchy, mild non-specific perifollicular inflammatory infiltrate within the upper dermis surrounding the follicular isthmus is noted. There is no interface dermatitis and no significant deep dermal inflammation. No fungal elements seen on the PAS stain.

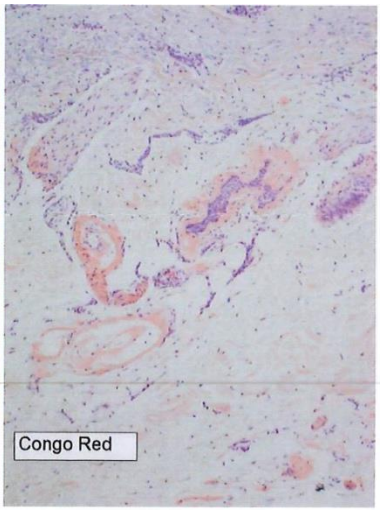
A   B

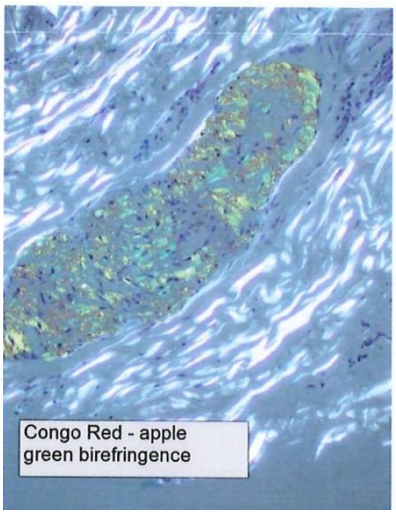
A: Marked decrease in hair follicles with prominent miniaturisation as well as telogen shift
B: No normal terminal anagen hairs are identified within the subcutis.

C   D

C/D: Arrows demonstrating pale amorphous eosinophilic material within blood vessel walls and within the connective tissue sheaths surrounding the hair follicles.







Amyloid can be demonstrated by the crystal violet stain, where the amyloid displays a purple-red metachromasia, However congo red staining is more specific, and displays the characteristic apple green birefringens when viewed under polarised light.

Discussion:

In addition to the special stains used for the demonstration of amyloid, further testing for kappa and lambda light chain immunohistochemistry was performed. The results showed a stronger signal for lambda in comparison to the kappa stain. Light chain associated amyloidosis (AL Amyloid) is one of the more common forms of amyloidosis and can often affect the skin, producing nodular or plaque like lesions. The alopecia in these patients can occur as the initial presentation of the systemic amyloidosis and or can occur as part of the disease process.

The literature (*Magro CM, et al 2019*) infer that the amyloid deposits can affect the hair growth cycle by various mechanisms such as compression of the pilosebaceous unit, replacement of the hair follicle by amyloid, and early entry to the telogen (quiescence) phase. Proteoglycans; dermatan sulfate, chondroitin 6 sulfate and heparan sulfate found in the connective tissue surrounding the hair follicles play a significant role in the hair growth cycle and are highly expressed during the anagen phase. Amyloid deposits found in this region alter and or can remove the function of these proteoglycans consequently leading to hair follicle involution and alopecia.

Adequate sampling and appropiate laboratory preparation is critical for an accuarate diagnosis of alopecia. Vertical sampling provides limited information as only approximately 10 percent of the follicles are demonstrated. This may not give an accurate respresentation of the disease process particurlarly if the pathological changes are present focally. Horizontal sampling enables the evaluation of follicular pathology even in focal disease. Furthermore, this type of sampling yeilds quantitative data of follicular cycling and complete assessment of the hair follicle from the bulb to the acroinfundibulum.

Acknowledgements:

This case was reported by Sullivan Nicolaides Pathology.

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Sectioning: A Deeper Look

There are numerous factors that must be considered when preparing tissue for IHC staining. Due to the diversity of tissue and media that may be involved, there is significant variability in sample preparation and no single answer for best practices. However, some key contributing factors can be identified. One oft-overlooked factor is sectioning thickness.

The amount of light permitted to pass through the tissue is critical for microscopic examination.[2] This is partially controlled by section thickness, which affects the contrast, sharpness, and morphological details of the tissue under the microscope, thus greatly influencing staining quality.[1]

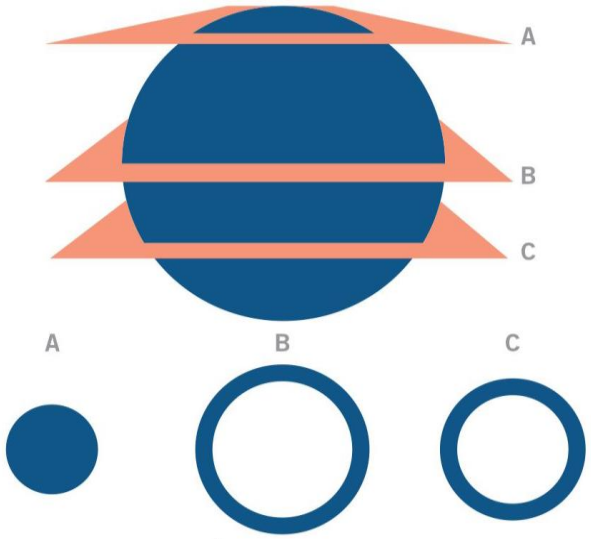
Generally, thicker sections demonstrate greater staining intensity due to more protein being present and labeled in a thicker three-dimensional structure. For example, a 7zwm section will have increased staining intensity compared to a 4µm section. In addition, what is visible in a 7µm-thick section may be lacking in a 4µm-thick section since less tissue is present.

Another area of consideration in sectioning thickness is how it affects the contents and composition of the block. Cells can range in size from 1 to 100 µm. In mammals, organelles and cellular components similarly vary in size. A ribosome may be 0.2 µm in diameter, while a cell nucleus may have a diameter of 6 µm. As a result, different-sized sections will better visually represent different cellular components, such as the membrane, cytoplasm, or nucleus.[1,2] Tissue sections >5 µm can produce more variation in staining intensity and make the assessment of cytoplasmic and membrane staining more complex than for nuclear staining.[3]

Tissue preservation and embedding medium should also be considered when determining section thickness.[1] Tissue is made firmer by the fixation process to preserve its structure and then may be infiltrated with a medium, such as wax or plastics, to support it, or may be fresh frozen. Section thickness typically ranges from 8-15 µm for frozen sections, 4–10 µm for wax sections, and 0.5–3 µm for plastic histological sections. For IHC staining, sections are cut between 3-5 µm.

Sectioning thickness is often overlooked as a factor in IHC staining outcomes but should always be considered in terms of tissue preservation, embedding medium, and staining quality.[1] At a minimum, consistency in sectioning is critical for the quality of patient care to prevent variability and confounding results. Sectioning thickness has significant implications in medical care, both now and in the future as digital imaging pathology becomes more prominent.[3]

Sectioning a sphere with a wall of finite thickness



DNA Helix 10 nm | Mitochondria 1 µm | Cell Nucleus 5-10 µm | Red Blood Cell 6 µm | Skin Cell 30 µm | Human Hair 100 µm | Blood Vessels 1 mm

Molecules — Organelles — Cells — Tissues

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IHC Antibody Review: Carbonic anhydrase IX (CAIX)

Org. No A0035235F

By Tu Anh Huynh

Carbonic anhydrase IX (CAIX) is a transmembrane protein and a member of the carbonic anhydrase family of enzymes. It plays a role in regulating pH, CO2 and HCO3 transport, and water and electrolyte balance. In recent years, CAIX has gained significant attention in the field of pathology due to its association with tumour hypoxia and potential as a diagnostic and prognostic marker in various cancers. Immunohistochemistry (IHC) is a powerful technique that leverages antibodies to visualize the expression and localization of proteins in tissue sections. CAIX antibody in IHC has become a valuable tool for pathologists, offering insights into tumour biology with some important considerations.

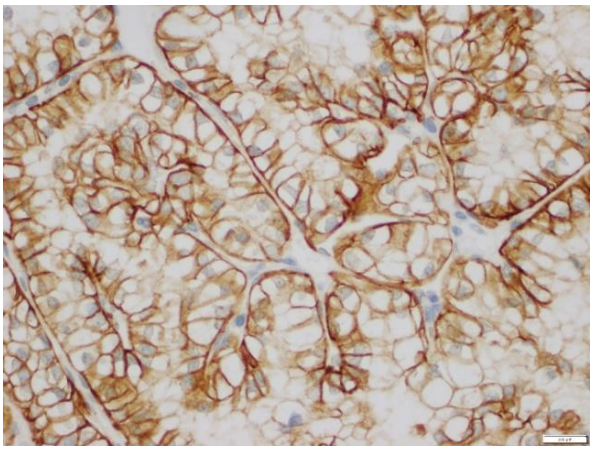

Fig. 1 - Kidney: Renal Clear Cell Carcinoma x40

CAIX expression is frequently upregulated in response to low oxygen conditions (hypoxia), a common feature of many solid tumours. IHC staining for CAIX can help identify hypoxic regions within tumours, which is crucial for understanding tumour aggressiveness and potential therapeutic strategies [1, 2].

CAIX expression can be helpful in differentiating between certain types of tumours. For example, in renal cell carcinoma (RCC), strong and specific CAIX expression is a characteristic feature of clear cell RCC, aiding in diagnosis [3].

In some cancers, high CAIX expression has been linked to poorer patient outcomes. This information can be valuable for patient risk stratification and treatment planning [2].

Choosing the right CAIX antibody is crucial, as some antibodies may exhibit cross-reactivity with other proteins, leading to false-positive results [2].

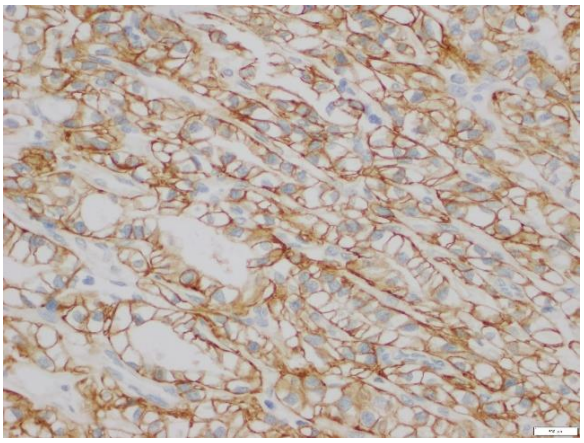

Fig 2 - Kidney: Renal Clear Cell Carcinoma x40

There is a need for standardized protocols for IHC staining with CAIX antibodies to ensure consistent and reliable results across different laboratories [4].

CAIX expression can vary significantly between different tumor types and even within the same tumor. It is important to interpret CAIX staining in the context of other clinicopathological features [3].

CAIX antibody in IHC offers a valuable tool for investigating tumor hypoxia, aiding in diagnosis, and potentially predicting patient prognosis in certain cancers. However, careful consideration of antibody specificity, standardized protocols, and the limitations of CAIX as a universal marker are essential for accurate interpretation and optimal clinical utility.

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Thank you to Ahida Batrouney from RMH for providing the images.

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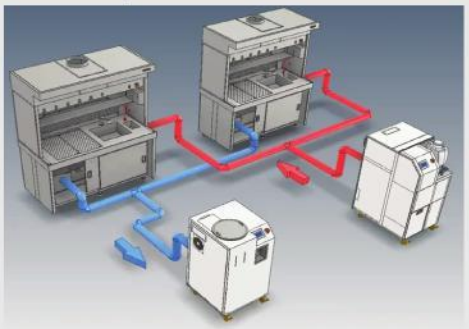

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Presenters: TBA

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Venue: Burnley Brewing
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Date: 9th-11th August 2024

National Histology Conference

Venue: Sydney, New South Wales

Presenters: TBA

Date: 5th Sep 2024

HGVT Scientific meeting

Topic: Student Presentation

Presenters: TBA