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PATHOLOGY FOCUS

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Issue 20

Medical Newsletter

Featured articles:

- Biopsy best practice
- ANCA-associated vasculitis
- Simplifying the new CPD changes

Season's Greetings

and Happy New Year!

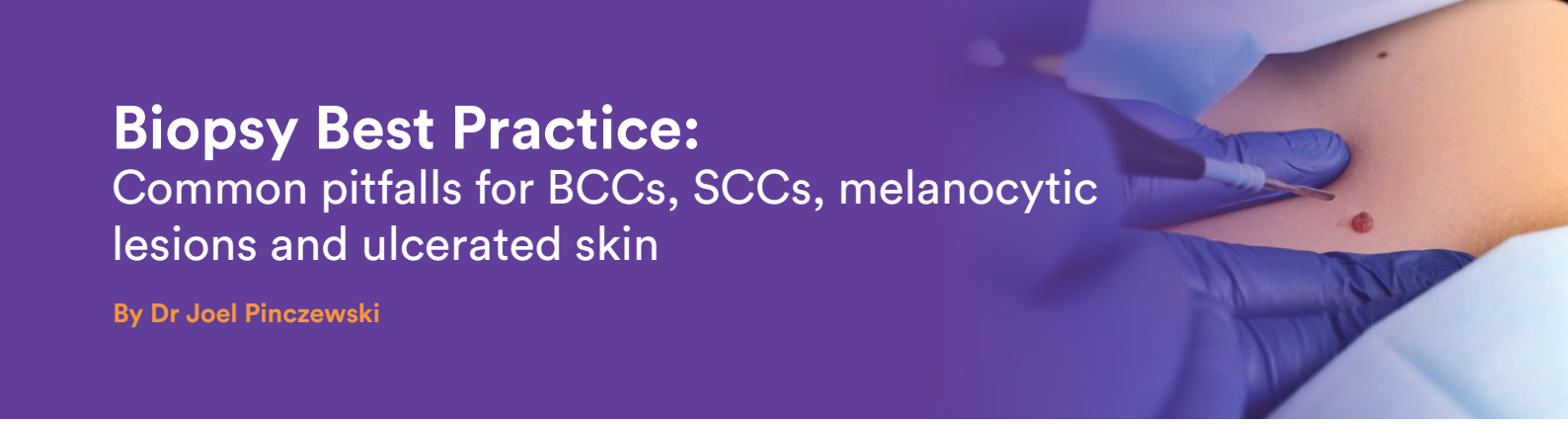
A heartfelt thank you from everyone at Clinical Labs

We would like to thank you for continuing to partner with Clinical Labs by allowing us to provide accurate and timely pathology results for your patients. We hope you enjoy a well-deserved rest with loved ones over the festive season, particularly after another challenging year for Australian healthcare.

Thank you for reading Pathology Focus this year — we hope you found our clinical content useful in your day-to-day practice, and we look forward to sharing with you even more insightful, relevant and topical articles from the world of pathology in 2023.

From everyone at Clinical Labs, we wish you a wonderful festive season and New Year filled with joy, happiness and good health, and we look forward to working with you next year.

Biopsy Best Practice:
Common pitfalls for BCCs, SCCs, melanocytic lesions and ulcerated skin



By Dr Joel Pinczewski

The clinical information and biopsy type that clinicians provide to pathologists can be critical in ensuring a correct diagnosis in skin biopsies. This is true for both neoplastic and inflammatory skin lesions. In previous articles my colleagues have provided some biopsy recommendations for different types of skin lesions. In this article, I will discuss diagnostic pitfalls that can occur when inadequate biopsies are provided as well as the importance of clinicopathologic correlation when interpreting skin biopsies.

Basal cell carcinoma

I will begin by discussing basal cell carcinoma (BCC). Basal cell carcinoma is the most common type of skin malignancy. It includes more aggressive and less aggressive subtypes, as shown in Table 1. Diagnosing a basal cell carcinoma is generally quite straightforward. However, there are histologic mimics of basal cell carcinoma and misdiagnoses can occur, particularly in the setting of small biopsies containing ulcerated skin or in very superficial biopsies.

For example, dermatofibromas can contain areas of basal cell hyperplasia (a type of benign reactive process). Basal cell hyperplasia typically does not pose a diagnostic dilemma for experienced dermatopathologists. However, less experienced pathologists do sometime mistake it for a basal cell carcinoma, particularly in very superficial biopsies. Collision tumours – a mixture of basal cell carcinoma and dermatofibroma – can also occur, so not all basaloid lesions occurring in dermatofibromas are benign. The RACGP website includes a case report of such a collision tumour[1].

Among the malignant lesions that can be mistaken for basal cell carcinoma, melanoma and merkel cell carcinoma are two examples. The prognosis and clinical management of both melanoma and merkel cell carcinoma are significantly different from basal cell carcinoma. Therefore, a misdiagnosis of either would have potentially profound clinical consequences for a patient.

Another issue with superficial biopsies is that they make it difficult to identify subtypes of basal cell carcinoma. This is because superficial biopsies often prevent assessment of architectural features in basal cell carcinomas. Therefore, for example, an infiltrative growth pattern may not be evident in a superficial biopsy. I often see examples of infiltrative basal cell carcinoma in excision specimens which were incorrectly classified as nodular type basal cell carcinoma in the original superficial shave biopsy of the lesion.

Table 1. BCC subtypes

Less aggressive subtypes	More aggressive subtypes
• Superficial & multifocal • Nodular • Fibroepithelioma of Pinkus • Infundibulocystic • Organoid • Adenoid • Pigmented	• Micronodular • Infiltrating • Metatypical • Sclerosing/morphoeic/ morphoeaform

The type of biopsy that a clinician provides can also be significant in diagnosing superficial and multifocal type basal cell carcinomas since this type of basal cell carcinoma has skip areas between nests of malignant cells, as shown in Figure 1. This means that a narrow punch biopsy may not contain malignant cells, particularly if the reporting pathologist has not examined multiple levels of tissue. The gaps between nests of malignant cells in superficial and multifocal basal cell carcinomas also makes it difficult to confirm margin status in narrowly excised lesions.

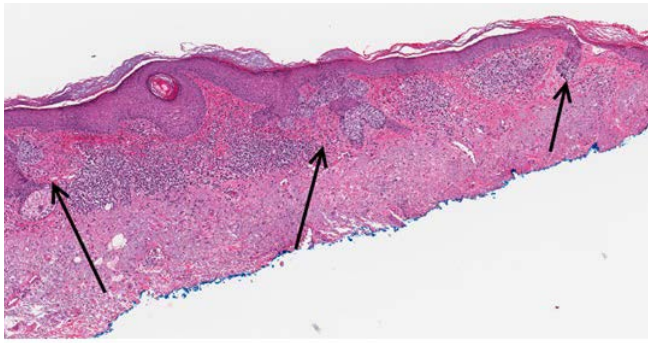


Figure 1. Superficial and multifocal basal cell carcinoma.

Squamous cell carcinoma

Another common type of skin cancer is squamous cell carcinoma (SCC). Well differentiated squamous cell carcinoma can be a difficult diagnostic area since various mimics can occur. Specifically, reactive atypia can occur in response to trauma, infections and inflammation and this can be extremely difficult to differentiate from a well differentiated squamous cell carcinoma in narrow punch biopsies or in superficial biopsies. An examination of the medical literature reveals various papers describing the difficulties in differentiating inflammatory lesions such as hypertrophic lichen planus from squamous cell carcinoma[2]. As a result, in inadequate biopsies, pathologists often use terminology such as “squamoproliferative lesion;

well differentiated squamous cell carcinoma cannot be excluded" or "atypical squamous proliferation" when they cannot confidently confirm or exclude a diagnosis of squamous cell carcinoma. In order to minimise these types of non-committal diagnoses, it is best to provide the pathologists with an adequately sized biopsy and to also provide adequate clinical information.

Melanocytic lesions

Melanocytic lesions are another area where the nature of the biopsy is critical for an accurate diagnosis. I recently received two separate cases where an atypical melanocytic lesion had been curetted by clinicians. Unfortunately one of these was a large invasive malignant melanoma. Curette biopsies of melanocytic lesions create diagnostic difficulties for the pathologist and prevent assessment of margins. Fortunately, most clinicians understand that curettage of atypical melanocytic lesions is to be avoided.

Some clinicians choose to do small punch biopsies of atypical melanocytic lesions and this can also cause diagnostic difficulties. Melanomas can arise in naevi and these may be missed due to sampling issues in punch biopsies. Also, punch biopsies often prevent assessment of symmetry and circumscription, two important factors used in differentiating benign from malignant melanocytic lesions. The preferred biopsy technique for atypical melanocytic lesions is a narrow shave excision.

> ***"The preferred biopsy technique for atypical melanocytic lesions is a narrow shave excision."***

Ulcerated skin

Another issue that occurs quite commonly is clinicians sending biopsies of ulcerated skin to evaluate for suspected inflammatory or neoplastic skin lesions. Ulceration can pose difficulties in evaluating for both types of lesions. Sending a small/partial biopsy of ulcerated skin can cause diagnostic difficulties in squamoproliferative lesions. For example, if the clinical impression is squamous cell carcinoma in situ (Bowen's Disease) then sending a biopsy of wholly ulcerated skin is not going to provide any useful diagnostic information. Ulceration can also pose diagnostic challenges in deeper lesions. This is because reactive atypia and epidermal hyperplasia can occur in the setting of ulceration, see Figures 2 & 3, and differentiating this from a well differentiated squamous cell carcinoma in a small/partial biopsy may be very difficult.

In regard to suspected inflammatory lesions, ulceration also causes diagnostic difficulty since reactive spongiosis and reactive inflammation typically occur in the vicinity of ulcerated skin. Clearly, there are some instances where taking a biopsy of ulcerated skin cannot be avoided, such as ulcerated solitary lesions. However, where possible, it is best not to biopsy ulcerated skin and especially not to send a biopsy containing only ulcerated skin without some intact epidermis.

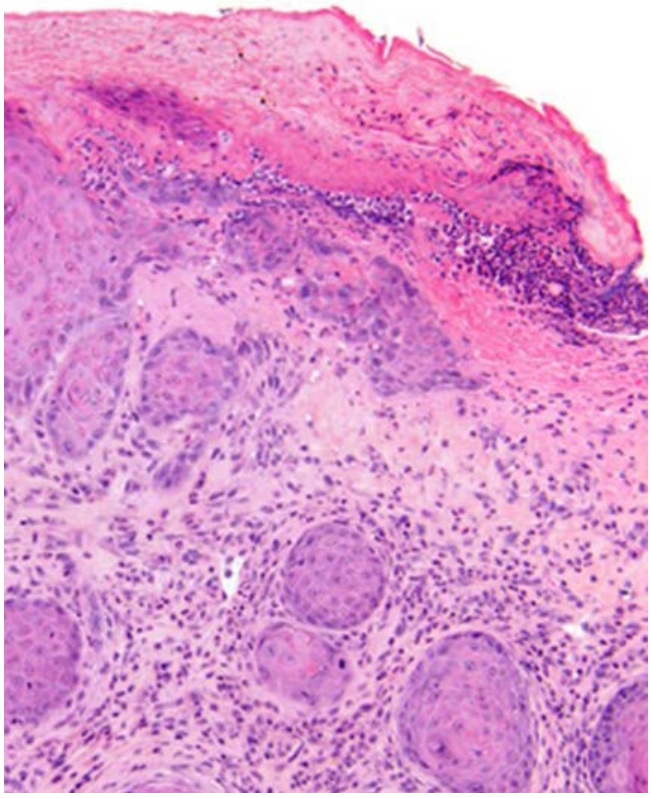


Figure 2. Is this lesion benign (reactive squamous hyperplasia) or it it a well differentiated squamous cell carcinoma?

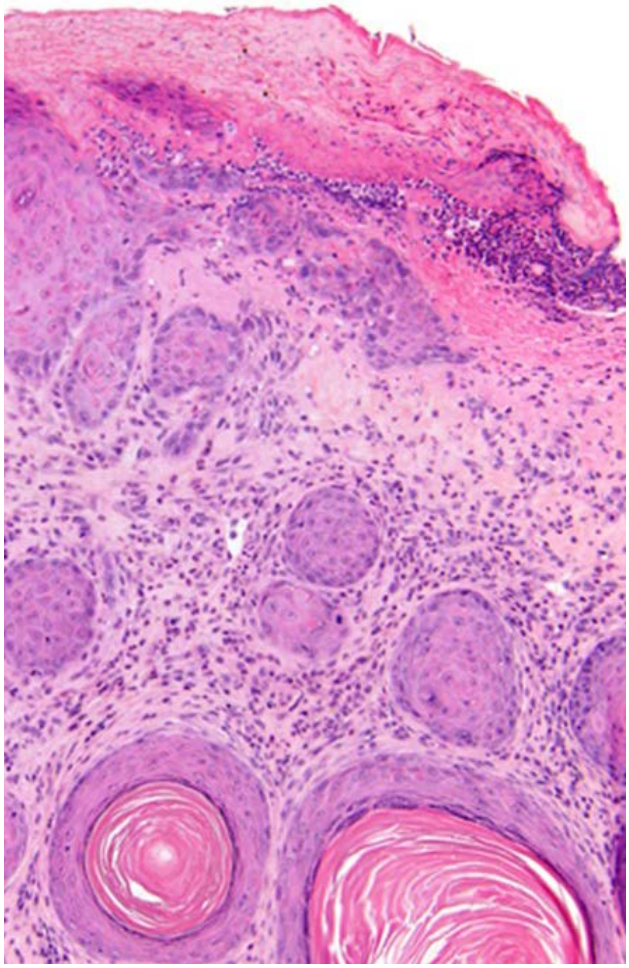
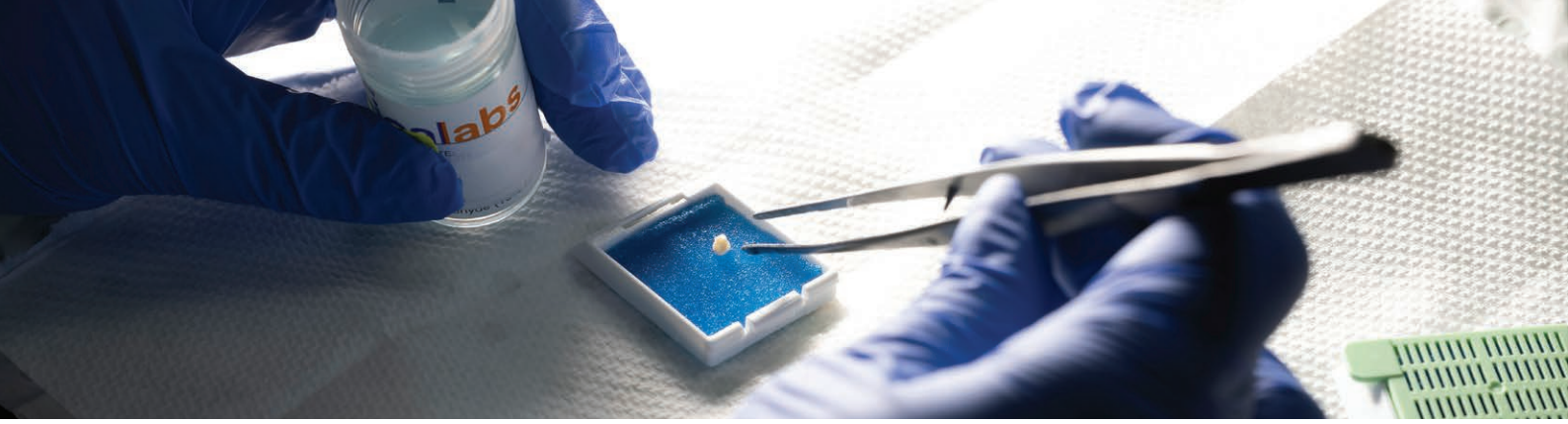


Figure 3. A deeper biopsy revels that this lesion contains reactive squamous proliferation.



Multiple biopsies

A couple of additional points that I would like to make are in terms of numbers of biopsies and types of biopsies. If an inflammatory lesion, an infection or a cutaneous lymphoma is suspected, then sending at least two biopsies is more likely to yield diagnostically useful findings and therefore an accurate diagnosis. This is because the findings in any one biopsy may not be representative due to sampling issues. For example, an insect bite reaction or some other incidental lesion may be sampled inadvertently. If an immunobullous disease, such as bullous pemphigoid is suspected or for suspected vasculitis, it is best to also include a biopsy of fresh peri-lesional tissue in saline soaked gauze or Michel's transport medium so that immunofluorescence staining can be performed.

The importance of providing adequate clinical information

Clinicopathologic correlation is also very important in many instances. Certainly in the setting of inflammatory lesions it is important to provide the pathologist with suitable and accurate clinical information. Please be careful when using terms like "rash". Some clinicians use this term very loosely. For example, it is not uncommon to receive a biopsy of a basal cell carcinoma with the clinical history of "rash". Please specify in the clinical information whether you suspect a lesion to be neoplastic or inflammatory in nature. Please also indicate whether the lesion you are sampling is solitary or whether the patient has multiple similar lesions. Other important information includes: the colour, size, shape, onset and duration of the lesion. For inflammatory lesions please indicate if there is any known association. For example, has the patient started a new medication. Please indicate if the lesion is painful or itchy. Clinical photographs can also be very helpful in some cases.

Adequate clinical information is also important when evaluating neoplastic skin lesions and in particular for melanocytic lesions. For example, if there is a known history of trauma please include this in the clinical information. Traumatisation can potentially explain atypical finding in a melanocytic lesion. This is because reactive atypia is a common feature of recurrent naevi. The atypia in recurrent naevi can closely mimic a malignant melanoma, particularly in a partially sampled melanocytic lesion. Diagnosing recurrent naevi can be difficult without adequate history in partially biopsied melanocytic lesions.

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Dr Joel Pinczewski obtained a PhD in virology from Monash University. Soon after he moved to the United States where he completed his postdoctoral training at the National Institutes of Health. Dr Pinczewski subsequently completed medical school at the Saba University School of Medicine and a residency in Anatomical and Clinical Pathology at the University of Maryland. He then completed a dermatopathology fellowship at the University of Virginia where he trained with two of the leading dermatopathologists in the United States. Dr Pinczewski is certified in Anatomic, Clinical and Dermatopathology by the American Board of Pathology. He is also a fellow of the Royal College of Pathologists of Australasia (FRCPA). Dr Pinczewski has published numerous articles in the medical literature. He is a passionate teacher and researcher and has delivered lectures in dermatopathology both nationally and internationally. Dr Pinczewski is skilled in all areas of dermatopathology.

ANCA-associated vasculitis

By Associate Professor Louise Smyth

History

In Europe in 1973, in a study of neutrophil-specific antibodies by indirect immunofluorescence (IIF) in patients with rheumatoid arthritis and Felty's syndrome, Allan Wiik detected antibodies demonstrating cytoplasmic granules of neutrophils that were present in a patient with crescentic glomerulonephritis.

The first published series of Anti-Neutrophil Cytoplasmic Antibody (ANCA) in renal disease appeared, in a short report in the BMJ in 1982, from Melbourne's St Vincent Hospital group – Davies, Moran, Niall and Ryan – who described the association of a serum factor (shown to be IgG) that demonstrated the cytoplasm of human neutrophils, by IIF, in eight patients with renal biopsy proven segmental necrotising glomerulonephritis with crescents, morphologically indistinguishable from microscopic polyarteritis nodosa. Viral infection was the suspected initiating event, and all cases resolved with treatment with only two reported recurrences (Davies, Moran, Niall, & Ryan, 1982).

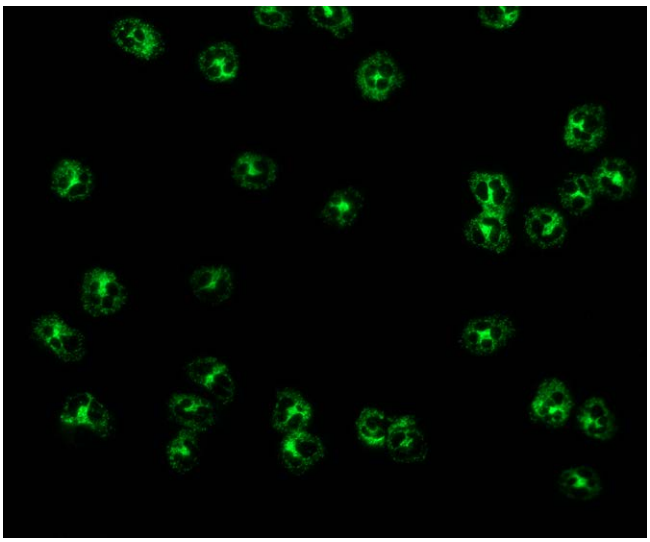
However, it would not be until 1985 that the diagnostic potential of testing for ANCA in Granulomatosis with Polyangiitis (Wegener's) [GPA] was defined in a series of European papers by Niels Rasmussen, Fokke van der Woude and others (Rasmussen, Wiik, & Jayne, 2015). The immunofixation techniques used either alcohol or formalin fixed neutrophils, resulting in two different effects on the distribution of antigenic substances in neutrophils. Using ethanol-fixed substrate, some substances are dissolved and migrate to a perinuclear position and attach to the nucleus. The predominant antigen, behaving in this manner and thus producing a perinuclear (p-ANCA) pattern, is *myeloperoxidase (MPO)*. This phenomenon means that when ANA is present, p-ANCA cannot be excluded. There are several other antigens present in neutrophil cytoplasm that behave in a similar manner: elastase, cathepsin G, azurocidin, lactoferrin, lysozyme, and bactericidal/permeability-increasing protein. *Proteinase-3 (PR3)* does not dissolve and remains within primary granules resulting in the classical c-ANCA (cytoplasmic) pattern. Antibody to LAMP-2 produces a similar pattern to PR-3, although some controversy exists regarding its clinical significance. The difference was codified and the standardisation of this method was agreed to for laboratory use at the first international workshop on ANCA in 1988 (Rasmussen, Wiik, & Jayne, 2015).

Table 1. Some ANCA associated disorders

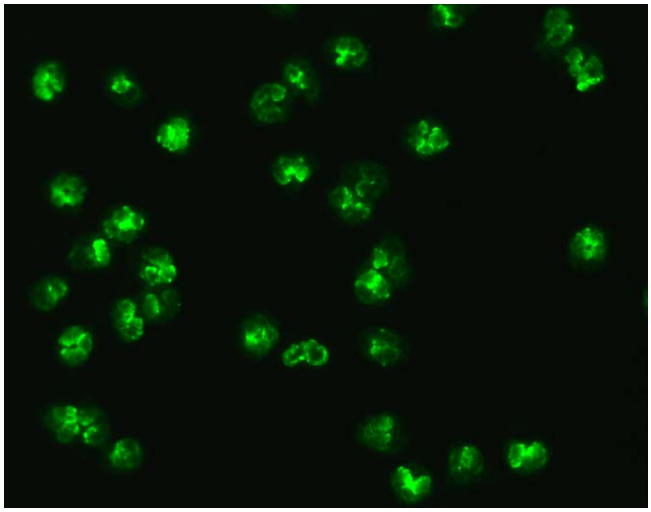
Disease	ANCA pattern	ANCA specificity
Vasculitis (Table 2)	C or P	PR3, MPO*
SLE		Variable
RA		Lactoferrin
IBD/Liver disease	Atypical	Variable, usually neither PR3 nor MPO
Endocarditis		PR3, MPO
Chronic infections		Variable, includes BPI
Haematopoietic malignancies		May be PR3 or MPO
Drugs		MPO, elastase or lactoferrin

*See Table 2.

Indirect Immunofluorescence, using ethanol-fixed substrate, remains the most frequent screening method for ANCAs, with follow up by specific, quantifiable tests for individual antibodies. In most (other than reference or research) laboratories this testing is limited to anti-PR3 and anti-MPO antibodies. Other ANCA are also seen in inflammatory bowel disease and liver diseases (predominantly in Ulcerative Colitis and Primary Sclerosing Cholangitis, respectively), in other systemic inflammatory conditions, in infective diseases, in malignancies, and in adverse drug reactions (Table 1). ANCA that are perinuclear in distribution and negative for anti-MPO or anti-PR3 have been designated x-ANCA.



c-ANCA.
https://commons.wikimedia.org/wiki/File:C_ANCA.jpg



p-ANCA.
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The vasculitides

Vasculitis refers to inflammation within the wall of blood vessels. There may be inflammation in the surrounding adventitia. The International Chapel Hill Consensus Conference on the Nomenclature of Systemic Vasculitides 2012 (CHCC2012), revised its 1994 consensus that set names and constructed definitions for the most common forms of vasculitis; it forms the basis for the ACR/EULAR classification and diagnostic criteria for the vasculitides (Jennette *et al*., 2013). The Chapel Hill Nomenclature does not include vasculitides of infectious origin. The vasculitides are divided by the size/type of the affected vessels and integrates aetiology, pathogenesis, pathology, demographics and clinical manifestations (Jennette *et al*., 2013). The major categories are:

- Large Vessel Vasculitis: Takayasu Arteritis, Giant Cell Arteritis
- Medium Vessel Vasculitis: Polyarteritis Nodosa, Kawasaki Disease
- Small Vessel Vasculitis
    - ANCA-Associated Small Vessel Vasculitis: GPA, MPA, EGPA
    - Immune Complex Small Vessel Vasculitis: Cryoglobulinaemic Vasculitis, IgA Vasculitis, Hypocomplementaemic Urticarial Vasculitis, Anti-GBM Disease
- Variable Vessel Vasculitis
- Single Organ Vasculitis
- Vasculitis Associated with Systemic Disorders
- Vasculitis Associated with Probable Aetiology.

Adapted from Jennette et al.

The pathogenesis of some vasculitides is still poorly understood, but the discovery of the ANCAs has driven much of the revision (Table 2). In both GPA and MPA there is strong clinical and experimental evidence for pathogenicity of the antibodies (Weiner & Segelmark, 2016) and thus a rationale for immunosuppressive therapy.

Table 2. ANCA in Vasculitis

Disease	ANCA specificity	Frequency
GPA	PR3	75%
Variable MPO association		
Europe	*MPO*	*10-20%*
Asia	*MPO*	*>50%*
MPA	PR3	20-30%
	MPO	60%
EPGA	PR3	5%
	MPO	+/- 40%
Anti-GBM disease	PR3	Rare
	MPO	~30-35%
IgA-vasculitis	IgA-ANCA	

GPA (granulomatosis with polyangiitis); formerly Wegener's granulomatosis), MPA (microscopic polyangiitis); formerly microscopic polyarteritis nodosa, and EGPA (eosinophilic granulomatosis with polyangiitis); formerly Churg–Strauss syndrome. Data adapted from Weiner & Segelmark (2016) and Austin *et al*. (2022).

In February 2022, American College of Rheumatology/European Alliance of Associations for Rheumatology Classification Criteria for Granulomatosis with Polyangiitis, Microscopic Polyangiitis and Eosinophilic Granulomatosis with Polyangiitis were released, and are available on the website of the American College of Rheumatology (https://www.rheumatology.org/Practice-Quality/Clinical-Support/Criteria#VasculitisClass).

Renal disease

Recent approaches to AAV are predicated upon the type of ANCA present as there is significant clinical overlap between the traditional diagnoses. Furthermore, while the majority of clinical phenotypes associate with either anti-PR3 or anti-MPO with some specificity, variable association occurs (Table 2). Additionally, ANCA may occur in anti-GBM Disease. ANCA, of either pattern or antigen association, also occur in idiopathic Rapidly Progressive Glomerulonephritis (RPGN), although late-stage crescentic disease may make classification more difficult.

In many cases quantitative results of, especially, the PR3 ANCA present corresponds with disease activity and can be used for disease monitoring and prognosis, in keeping with their role as a pathogenic antibody, especially in prediction of relapse. In renal disease, the outcome and likely antibody vary between younger and older patients, with corresponding differences in disease profile. While the risk of end-stage renal failure has been shown to be higher for PR3- positive disease and overall renal involvement to be greater in those who are MPO-positive, the Diagnostic and Classification Criteria for Primary Systemic Vasculitis (DCVAS) study showed that older patients (>65 years) are more likely to develop disease associated with MPO-ANCA, and succumb within 6 months (Austin *et al*., 2022). Anti-GBM Disease has been shown to have a poorer outcome when ANCA is present in addition to anti-GBM antibody.

Conclusion

ANCA are important diagnostic, prognostic and disease monitoring tools that are especially useful in the management of small vessel vasculitis in pauci-immune necrotising and crescentic glomerulonephritis. Several other specificities of ANCA than anti-PR3 and anti-MPO occur in a variety of clinical disorders and may provide useful information for clinicians, especially in the setting of Inflammatory Bowel Disease and related liver disease.

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Simplifying the new CPD changes:

Clinical Labs offers activities to complete all 50 required hours

New CPD requirements for the 2023-2025 triennium

As of 1 January 2023, the Medical Board of Australia (MBA) requires all Australian GPs to complete the following continuing professional development (CPD) requisites:

- Log 50 hours of CPD every year
- Complete a professional development plan (PDP) every year
- Refresh your skills with one CPR course during the triennium

There are now three different types of activities you'll need to complete to gain the required 50 CPD hours each year.

RACGP CPD Minimum Requirements for 2023 - 2025 Triennium (50hrs per year)

5hrs	5hrs	12.5hrs	12.5hrs	15hrs
Reviewing Performance	Measuring Outcomes	Educational Activities (Knowledge and Skills)	Any Activity Type	Reviewing Performance and/or Measuring Outcomes

Education Activities: activities that expand your General Practice knowledge and skills. Examples - Reading educational material (articles, journals), Workshops, Conferences, Lectures/Webinars.

Measuring Outcomes: activities that use your work data to ensure quality results. Examples - Audits, Practice Accreditation, Development of clinical guidelines.

Reviewing Performance: activities that require reflection on feedback about your work. Examples - Case based discussions, Peer group learning, Patient feedback.

How Clinical Labs' CPD Programs work with the new requirements

Diabetes Clinical Evaluation Program

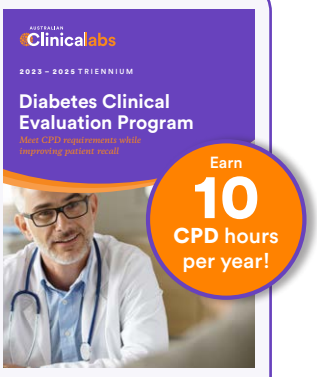
A CPD clinical audit designed to help you easily manage and provide clinical care for your patients living with diabetes.

Annual Criteria for CPD Program Qualification

- Patients with diabetes referred for HbA1c analysis (minimum 40 episodes)
- Minimum 4 program views/logins

- Minimum 12 months of registration
- Reflection activity completed

Scan the QR code to register for the 2023-2025 triennium.



5hrs	5hrs	12.5hrs	12.5hrs	15hrs
Reviewing Performance	Measuring Outcomes	Educational Activities (Knowledge and Skills)	Any Activity Type	Reviewing Performance and/or Measuring Outcomes

Skin Excision Evaluation Program

A CPD clinical audit that delivers a truly educational experience by analysing your diagnostic skill for identification of high-risk lesions.

Annual Criteria for CPD Program Qualification

- 40 histological samples submitted on the specific audit request forms

- Minimum 12 months since registration
- Reflection activity completed

Scan the QR code to register for the 2023-2025 triennium.

5hrs	5hrs	12.5hrs	12.5hrs	15hrs
Reviewing Performance	Measuring Outcomes	Educational Activities (Knowledge and Skills)	Any Activity Type	Reviewing Performance and/or Measuring Outcomes

If you complete both Clinical Labs CPD programs

5hrs	5hrs	12.5hrs	12.5hrs	15hrs
Reviewing Performance	Measuring Outcomes	Educational Activities (Knowledge and Skills)	Any Activity Type	Reviewing Performance and/or Measuring Outcomes

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