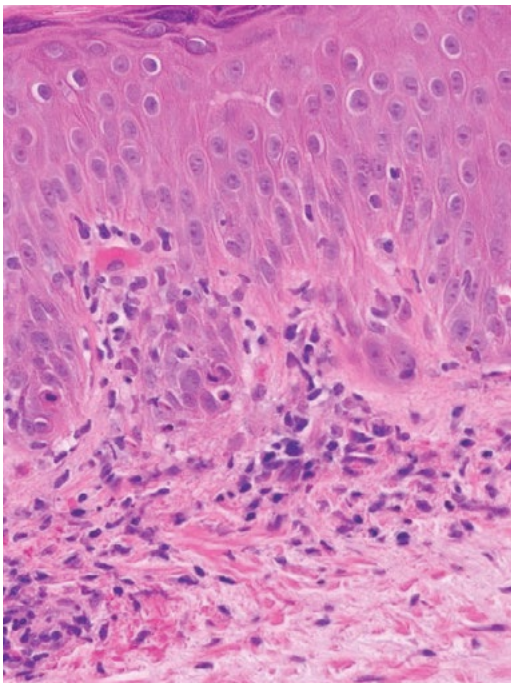

The red herring in infective serology

Miranda Wallace, Jenny Robson, Jim Muir

CASE

A man aged 75 years, who had just returned from Thailand, presented with a three-day history of an asymptomatic rash after a recently resolved flu-like illness. He denied sexual contact, medication or recreational drug use, or insect bites. He had no significant past medical history.

He was afebrile with no constitutional symptoms. There was a widespread eruption most severe on his lower limbs with discrete papules, which in areas were becoming confluent. Below the knees, the eruption was markedly purpuric but blanched completely elsewhere on the body (Figure 1).

QUESTION 1

Is this palpable, purpuric rash vasculitis? Why should vasculitis be excluded?

QUESTION 2

Is a biopsy warranted? Which investigations should be ordered? How will these inform the differential diagnosis for the rash?

QUESTION 3

Which differentials need to be considered in a returned traveller from Thailand with a rash? Which need to be excluded?

QUESTION 4

What would you conclude from positive Ross River virus (RRV) immunoglobulin G (IgG)/immunoglobulin M (IgM) serology?

ANSWER 1

Purpura raises the concern of vasculitis. This feature limited to below the knee makes vasculitis less likely. Red cell extravasation in inflamed skin can be secondary to gravitational factors and/or disordered coagulation. Purpura is a hallmark of vasculitis and would thus be a feature in all locations if this was the diagnosis.

Vasculitis still needs to be excluded as systemic involvement is possible.

ANSWER 2

The likely differential diagnoses for the rash are morbilliform drug reaction, viral/infective exanthem or vasculitis. A biopsy would help exclude vasculitis and might show

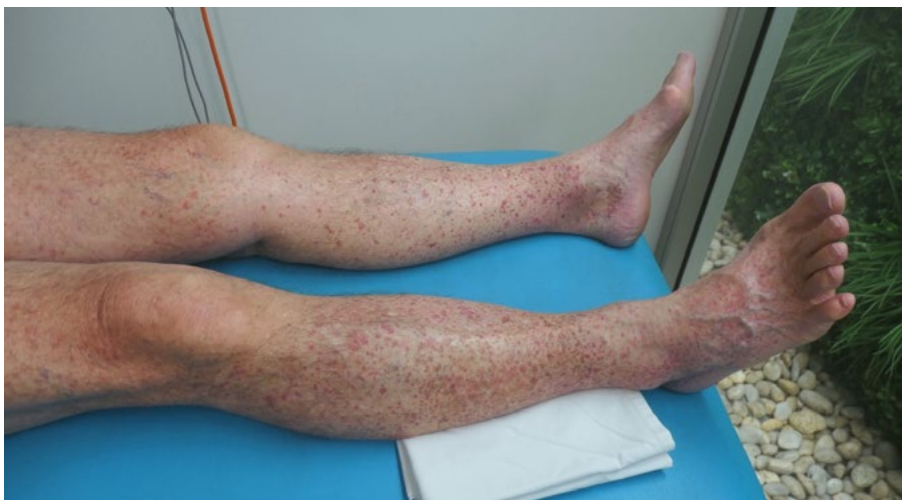

Figure 1. Lower limb rash with purpura below the knee.

features to support one or other of the main differentials (Figure 2).

The following investigations tests were ordered:

- full blood count (FBC) ± blood film
- electrolyte, urea, creatinine (EUC), liver function test (LFT), c-reactive protein (CRP), erythrocyte sedimentation rate (ESR)
- hepatitis B surface antigen (HBsAg) and antibody (HBsAb)
- hepatitis C antibody total (HCV)
- HIV antibody and/or p24 antigen (HIV Ag/Ab)
- Ross River virus antibodies (RRV IgG and IgM)
- dengue antibodies (IgG and IgM) and dengue virus NS1 antigen (NS1 ag)
- chikungunya immunofluorescence assay (IF), IgG and IgM
- Zika virus antibodies (ZIKV IgG and IgM)
- syphilis serology (*Treponema pallidum* IgG)
- cytomegalovirus antibodies (CMV IgG and IgM).

It is important to consider the endemicity of infections in direct testing. Further serology can be requested if needed.

ANSWER 3

Arthropod-borne diseases such as dengue, Zika, chikungunya and rickettsial infections, particularly scrub typhus, are endemic in Thailand.[1] Common conditions are cytomegalovirus (CMV), Epstein–Barr virus (EBV), streptococcal infections, various vaccine-preventable diseases, including rubella and measles, drug reactions, haematologic disorders and vasculitis (Table 1).

The distribution and type of rash dictates the rationalisation of tests. Very rarely will EBV produce a secondary immune thrombocytopaenic purpura that might present as lower limb purpura.

ANSWER 4

The RRV IgG was positive, with symptoms present for only one week. IgG levels are usually positive at least two weeks after the IgM level becomes detectable, making this early IgG positivity likely from a past infection with persistent IgM.

A positive IgM (IgM+) might represent a nonspecific cross-reaction or be secondary to another infection such as EBV, CMV or arbovirus.[3] Repeat sera collected 10–14 days apart will detect changing antibody levels, particularly where there is IgM cross-reactivity.

RRV is the most common mosquito-borne disease in Australia[4] and although outbreaks have occurred in the Pacific, it is not endemic to Thailand. This makes acute RRV infection less likely.

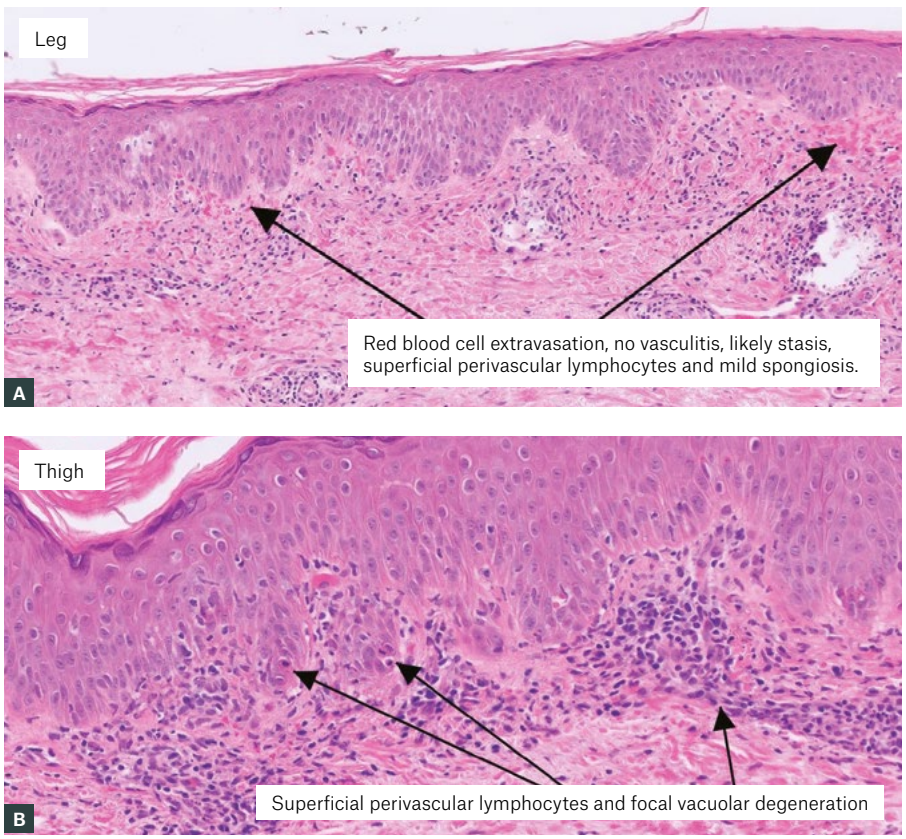

Figure 2. Histopathology of limb biopsy. **A**. Lower leg: the biopsy of the leg shows red blood cell extravasation, no vasculitis, likely stasis, superficial perivascular lymphocytes and mild spongiosis. The lower limb is purpuric, but not vasculitic. This emphasises the effect of site on red cell extravasation, likely due to gravitational stasis. **B**. Upper thigh: the biopsy of the thigh shows superficial perivascular lymphocytes and focal vacuolar degeneration. There is minimal to no red cell extravasation on the upper thigh, compared to the lower leg shown in Figure 2A.

CASE CONTINUED

Full blood count demonstrated a mild leukopenia and neutropaenia, thrombocytopaenia and a mildly raised aspartate transaminase. Additional serology results were received after the initial positive RRV IgM/IgG (Table 2) and histopathology (Box 1).

QUESTION 5

What is the most likely diagnosis now?

QUESTION 6

Why is confirming dengue important? How is 'true' dengue serology determined?

ANSWER 5

Biopsy has excluded vasculitis and supports a viral exanthem. The red blood cell extravasation is greater in the lower leg biopsy. This is likely gravitational with exacerbation by the mild thrombocytopaenia. IgM positivity to RRV, chikungunya and

Table 1. Differential diagnoses of infectious causes of rash in a returned traveller

Infectious causes	Incubation period[2]	Vaccine?	Notifiable disease?[A]
Malaria	7–30 days	Yes	Yes
Measles[B]	7–18 days	Yes	Yes
Rubella	14–17 days	Yes	Yes
EBV	4–6 weeks	No	No
CMV	3–12 weeks	No	No
Chikungunya	3–11 days	Yes (FDA approved, not available in Australia)	Yes
Dengue fever	4–7 days	No (not if initial infection)	Yes
BFV	7–10 days	No	Yes
RRV	3–9 days	No	Yes
Streptococcal disease[B] (GAS)	1–3 days	No	Yes
Enterovirus	3–5 days	No	No
Rickettsial disease	2–14 days	No	Yes
Acute HIV[B]	2–4 weeks	No	Yes
Parvovirus B19	3–7 days	No	No
Syphilis	10–90 days	No	Yes

[A]Cases that are required to be reported to the Australian National Notifiable Disease Surveillance System.

[B]Infections that are crucial not to miss.

BFV, Barmah Forest virus; CMV, cytomegalovirus; EBV, Epstein–Barr virus; GAS, group A streptococcus; HIV, human immunodeficiency virus; RRV, Ross River virus.

dengue is present. Positive non-structural protein 1 (NS1) confirms dengue viral presence, as does dengue serotype-1 polymerase chain reaction (PCR). Dengue-positive IgM without dengue IgG in this acute sample indicates likely primary, not secondary, dengue.

ANSWER 6

It is important to confirm a diagnosis of dengue because of the similarity of its presentation with other vector-borne viral diseases, and to rule out other conditions that might require specific intervention.

Reinfection due to different dengue serotypes (1–4) can occur, with secondary dengue often having a more severe clinical course.[5]

In primary infection, NS1 antigen is produced from days 1 to 9 after the onset of symptoms.[6] Detectable levels of IgM antibody will be produced five days after symptom onset, peaking within two weeks, followed by rapid decay, but might remain detectable for six or more months.[7] Dengue-positive IgG (dengue IgG+) indicates seroconversion. NS1 antigen early in infection paired with IgM+ dengue antibody is most suggestive of an acute dengue infection.[8]

Dengue is the commonest cause of fever in travellers from tropical countries.[9] Diagnosis is helpful in directing supportive treatment and guides surveillance strategies to inform public health control, and future vaccination.

Key points

- A palpable, purpuric rash is not always vasculitis.
- Cross-reactivity might exist among viruses, particularly among the various flavi and alpha viruses.
- Positive serology does not always indicate current acute infection.

Authors

Miranda Wallace BBioMedSc, MBBS MS, Associate Clinical Lecturer, School of Medical Sciences, Faculty of Medicine and Health, University of Sydney, Sydney, NSW

Jenny Robson MBBS, FRACP, FRCPA, FACTM, Pathologist-in-Charge, Department of Microbiology and Molecular Pathology, Sullivan Nicolaides Pathology, Brisbane, Qld

Jim Muir MBBS FACD, Head of Department, Department of Dermatology, Mater Hospital, Brisbane, Qld; Dermatologist, South East Dermatology, Brisbane, Qld; Associate Professor, Faculty of Medicine, University of Queensland, Brisbane, Queensland

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Correspondence to:
muirmed@outlook.com

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Table 2. Case serological results

RRV IgG (EIA)	Positive
RRV IgM (EIA)	Positive
Chikungunya IgG (EIA)	Positive
Chikungunya IgM (EIA)	Positive
NS1 (ICT/EIA)	Positive/Positive
Dengue IgG (EIA)	Negative
Dengue IgM (EIA)	Positive
Dengue PCR	Dengue 1 serogroup
Zika IgG (EIA)	Negative
Zika IgM (EIA)	Negative
BFV IgG (EIA)	Negative
BFV IgM (EIA)	Negative
Flavivirus[A] IgG (MIA)	Nonreactive
Flavivirus[A] IgM (MIA)	Reactive
Alphavirus[B] IgG (MIA)	RRV IgG reactive
Alphavirus[B] IgM (MIA)	RRV IgM reactive

[A]Includes dengue 1–4, JEV, MVE, Kunjin, Alfuy, Kokobera, Stratford, yellow fever, Zika.

[B]Includes RRV, BFV, Sindbis, chikungunya.

EIA, enzyme immunoassay; ICT, immunocapture lateral flow assay; IgG, immunoglobulin G; IgM, immunoglobulin M; MIA, microsphere; MVE, Murray Valley encephalitis; NS1, non-structural protein 1; PCR, polymerase chain reaction; POC, point of care; RRV, Ross River virus.

Box 1. Histopathology report

Punch biopsy sites

1. Left arm
2. Left upper thigh
3. Left lower leg

Microscopic examination of specimens

Specimens 1 and 3 (arm and upper thigh)

The specimens marked 1 and 3 show features consistent with a viral exanthem. There is mild hyperkeratosis and mild epidermal hyperplasia with focal vacuolar degeneration. The dermis shows a superficial perivascular lymphocytic inflammatory infiltrate with minimal red blood cell extravasation. Eosinophils are not a prominent feature and there are no features to suggest a vasculitis. The PAS stain is negative and there is no haemosiderin with the Perls' stain.

Specimen 2 (lower leg)

Sections show similar features as described above, apart from the fact there is slightly more red blood cell extravasation and some haemosiderin within the papillary dermis. The latter features might be due to co-existent mild stasis. There is no evidence of leukocytoclastic vasculitis.

PAS, periodic acid-Schiff.

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correspondence **ajgp@racgp.org.au**