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SARS-CoV-2 IgM testing for travellers: a private pathology perspective from New South Wales and the Australian Capital Territory, Australia

To the Editor,

We read with interest the letter by Hasan *et al.*[1] outlining the pitfalls of relying on SARS-CoV-2 IgM in a risk stratification matrix required prior to travel by some overseas countries (Table 1).[2,3]

The diagnosis of acute COVID-19 relies on SARS-CoV-2 nucleic acid amplification testing (NAAT).[4] However, since 8 November 2020, the Chinese Embassy has required both SARS-CoV-2 NAAT and SARS-CoV-2 IgM serology ('dual test') be performed within 48 hours of travel from Australia to China. We agree with Hasan and colleagues that IgM detection prior to travel has currently a low sensitivity of detecting cases that are polymerase chain reaction (PCR) negative yet potentially infectious. Conversely, IgM is frequently positive beyond the infectious period and the requirement for a negative 'dual test' prior to travel is unnecessary.

Our laboratories currently use two assays that have the ability to detect IgM: the Roche Elecsys Anti-SARS-CoV-2 assay that detects nucleocapsid IgM, IgA and IgG antibody (Roche Total; Roche, USA), and the Abbott Architect SARS-CoV-2 IgM assay that detects spike antibody (Abbott IgM; Abbott, USA). These two commercial in-laboratory tests (as opposed to rapid lateral flow tests) in our in-house validation studies were found to have similar high specificities (99.5% and 98.9%, respectively) to those stated by the manufacturer (99.8% and 99.56%). Sensitivities were found to be lower (67% for both Roche Total and Abbott IgM for samples that were 8–14 days post onset of symptoms in confirmed COVID cases) than those stated by the manufacturer (85% and 86%). This may relate to the greater proportion of mildly symptomatic patients that were included in our analysis when compared to that of the manufacturer and has been described previously.[5,6] The positive and negative concordance of the IgM also correlated reasonably well to immunofluorescence assay (IFA) IgM (78%) and Euroimmun IgA (68%) assays. While the Abbott IgM assay was positive in three cases where the IFA was negative, these were deemed to be true positives based on timing of confirmed infection. Therefore, both the Abbott IgM and Roche Total assay were found to have a low risk of false positive IgM results.

Table 1 Criteria for boarding[2,3]

Destination country	PCR	Serology	Further testing may be required
China	Negative PCR	IgM −	IgM +
Samoa	Negative PCR	IgM −	IgM +/IgG −
		IgG +	IgM +
		IgM −/IgG −	IgG −
		IgM +/IgG +	
		IgM −/IgG +	

We undertook a retrospective audit of all serology performed in our two laboratories between 9 July 2020 and 19 September 2021 with a particular focus on those requiring evaluation prior to travel.

A total of 5831 samples had COVID serology performed through our laboratories with 545 (9%) specifically for pre-travel testing. Sufficient history was provided in 504/545 (92%) to determine the country of destination. Of these, 224/504 (44%) were travelling to China. The next largest groups were flying to Samoa (n=59, 12%), USA (n=40, 8%), UK (n=26, 5%) and India (n=11, 2%).

The Abbott IgM assay was performed on 444 samples, while the Roche Total was performed on 152 samples; 101 of these samples were tested only with the Roche Total assay while the remaining 51 samples had Roche Total performed in addition to the Abbott IgM assay.

There were 45/444 (10%) positive Abbott IgM samples (with 44/44 returning a negative SARS-CoV-2 NAAT result from simultaneously collected nose and throat swabs), and there were 4/152 (3%) positive Roche Total samples, with 3/4 positive Roche samples also positive on the Abbott IgM assay, consistent with recent past infection with or without recent vaccination.

Of the 224 travellers to China, 15/190 (8%) were found to have positive Abbott IgM results with one of these also positive on the Roche assay and having confirmed infection overseas 6 months prior. This patient was eventually cleared for travel when a negative IgM result by IFA was obtained. Of the remaining 14, 10 were cleared to travel following reporting of the subsequent reflex Roche Total result which returned negative results for all of these samples. The remaining four that did not undergo reflex testing were reported as positive with further discussion with the Chinese Embassy allowing clearance due to negative PCR and recent vaccination in the previous 6 weeks considered the likely cause of the positive Abbott IgM result. Retrospective reflex testing found these four samples to also be negative on the Roche assay excluding recent infection. The remaining 34 travellers who were tested by the Roche Total alone were all negative. Since review of this dataset, an additional asymptomatic traveller to China was found to be positive by both Abbott IgM and Roche Total during the Delta outbreak in Sydney. He had not been vaccinated and, while PCR was negative, he was judged to have had recent infection. Travel was deferred and he was required to undergo repeat PCR testing (result on day 2 and day 22 negative), chest imaging (result normal) and repeat serology (3 weeks later: rising IgM and IgG levels) and travel was further delayed until his 'dual test' was negative.[7]

Of the 59 travellers to Samoa, 2/42 (5%) and 0/19 were positive on the Abbott IgM and Roche Total assays, respectively, with all reported as negative (following reflex

testing of the two Abbott IgM positive samples returning negative Roche Total results).

To further explore the likelihood of IgM positivity following vaccination, a random sample of 63 asymptomatic predominantly laboratory staff who had been recently vaccinated volunteered to be tested by the Abbott IgM assay. Positive IgM results were obtained in 24/63 (38%). Of these, 23/24 were obtained following vaccination with Pfizer Comirnaty with one following Astra Zeneca vaccination (Table 2), although due to different collection schedules it is difficult to compare the difference between IgM responses following the two vaccines in our tested cohort.

Because of the ever increasing vaccination rates in our community coupled with impending opening of the borders for overseas travel, it is expected that more patients will require serology testing prior to travel. Based on our data, a majority of these might be expected to return positive anti-spike IgM results (up to 75% 3 weeks post the first dose of Pfizer in our cohort) with IgM detectable up to 8 weeks post mRNA vaccination in one study.[8] Recently, travellers to China therefore have been required to have nucleocapsid-specific IgM tests to at least remove those who potentially only have vaccine-induced positive IgM results.

However, COVID cases have recently increased to high levels globally due to Omicron, and it is predicted that more COVID infections will develop as elimination strategies are abandoned. Serology testing prior to travel may also result in positive nucleocapsid IgM results from actual recent infection rather than vaccination and delay travel as we saw with the recent case described above. Others have also reported concerns about false positive IgM results that have delayed travel.[9,10] Of concern is the median time to IgM seroreversion in one study[11] being 7 weeks following infection, similar to the 53% of confirmed cases still IgM positive in the 7th week post-onset of illness in another study.[12] Therefore, it is expected that significantly delayed travel following actual recent infection will be likely for a large proportion of recently infected (but not infectious) travellers if this strict requirement is maintained.

In conclusion, particularly for travellers to China, it is probable that positive IgM results related to recent vaccination or infection will only become more common. The use of a nucleocapsid based assay such as the Roche Total assay may at least reduce the proportion of recently vaccinated travellers with positive IgM results. Such an assay can be utilised as a screening assay to avoid detection of vaccine-induced spike IgM. However, an increasing number of people have been infected with SARS-CoV-2 and therefore will have nucleocapsid antibodies detectable with the Roche Total assay. In our laboratory, these are then tested with a lateral flow nucleocapsid-specific IgM assay (due to unavailability of an automated IgM-specific nucleocapsid assay at time of writing) and if positive is likely to lead to significant travel delays. Thus, the pitfalls of IgM testing have major practical implications in terms of the inconvenience and expense of postponed travel and further testing, though these requirements are unlikely to change in the foreseeable future given that they have already remained in place for the last 15 months. Like others, we can only hope that the requirement for a negative 'dual test' is abandoned and NAAT results alone will continue to be the best guide to whether an individual traveller poses an infection risk.

Table 2 Patient characteristics of post-vaccination cohort

	Pfizer post dose 1 (median 21 days) *n*=8 (%)	Pfizer post dose 2 (median 42 days) *n*=45 (%)	Astra Zeneca post dose 1 (median 47 days) *n*=10 (%)
IgM positive	6 (75)	17 (38)	1 (10)
IgM negative	2 (25)	28 (62)	9 (90)
% female	7 (88)	34 (76)	5 (50)
Mean age, years	47.1	42.2	59.1
Previous COVID	0	2	6

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Vicious cycle of hypertriglyceridaemia and hyperglycaemia in an atypical case of lipoprotein lipase deficiency

To the Editor,

Severe hypertriglyceridaemia, defined as plasma triglycerides >10 mmol/L,[1] is primarily polygenic and contributed by multiple acquired factors including diet, obesity, alcoholism, diabetes mellitus, hypothyroidism, paraproteinaemia and drugs.[2] On the other hand, familial chylomicronaemia syndrome refers to a group of rare disorders of hypertriglyceridaemia, the most common being lipoprotein lipase deficiency (MIM #238600) due to pathogenic variants in the *LPL* gene (MIM *609708), with an estimated prevalence of 1 in 1,000,000. It is an autosomal recessive disorder characterised by impaired plasma clearance of both chylomicrons and very low-density lipoproteins. The majority of patients present before 10 years of age with recurrent pancreatitis secondary to severe hypertriglyceridaemia.[3]

We report an atypical case of lipoprotein lipase deficiency, with the patient presenting in late middle age without any history of pancreatitis or recurrent abdominal pain.

The patient, then a 56-year-old man, was admitted for transient ischaemic attack in July 1999. Fasting blood tests showed triglycerides 28.8 mmol/L, total cholesterol 8.2 mmol/L and glucose 6.4 mmol/L. Apart from impaired fasting glycaemia, no other secondary causes of dyslipidaemia were identified. He had no family history of hypertriglyceridaemia. Moderate hypertriglyceridaemia persisted after diet modification and gemfibrozil, which was started in September 1999.

At 62 years of age, he was diagnosed with type 2 diabetes mellitus which was initially managed with diet modification alone. Both his glycaemic control and hypertriglyceridaemia gradually worsened. Metformin and atorvastatin were started at 67 years of age, and gliclazide was added at 72 years of age. Two years later, both his HbA1c (6.1%) and plasma triglyceride level (2.3 mmol/L) reached a nadir.

However, his disease control deteriorated afterwards. At 77 years of age, his HbA1c increased to 9.2% despite increasing dose of metformin and gliclazide, and plasma triglycerides increased to 42.6 mmol/L despite concurrent use of rosuvastatin, fenofibrate and ezetimibe. Therefore, he was admitted for management of severe hyperlipidaemia. Repeat blood tests showed triglycerides 21.2 mmol/L, total cholesterol 10.6 mmol/L, HDL cholesterol 0.5 mmol/L, direct LDL cholesterol 1.7 mmol/L, apolipoprotein A1 1.11 g/L (reference interval 1.10−1.80 g/L) and apolipoprotein B 1.68 g/L (0.49−1.15 g/L). Lipoprotein electrophoresis showed type V hyperlipidaemia with dense chylomicron and VLDL lipoprotein bands. Insulin was started which improved both his glycaemic control and lipid profile. He was discharged a week later. His plasma triglycerides and HbA1c levels are summarised in Fig. 1.

A monogenic cause of hypertriglyceridaemia, in particular lipoprotein lipase deficiency, was suspected because of the disease severity and resistance to treatment. Sanger sequencing of the *LPL* gene (reference sequence NM_000237.2) revealed apparent compound heterozygosity for two previously reported pathogenic variants, c.292G>A p.(Ala98Thr) (dbSNP rs145657341) and c.835C>G p.(Leu279Val) (dbSNP rs371282890).[4,5] The diagnosis of lipoprotein lipase deficiency was thus confirmed. Cascade screening for at-risk family members was advised, and they preferred biochemical screening to genetic testing. Further history revealed that the patient was taking fish oil supplements which are contraindicated in lipoprotein lipase deficiency. Those supplements were subsequently stopped.

There is no known genotype-phenotype correlation in lipoprotein lipase deficiency.[3] The *LPL* Ala98Thr variant decreases both the secretion and the catalytic activity of lipoprotein lipase, although some residual activity is preserved. On the other hand, the *LPL* Leu279Val variant almost abolishes the catalytic activity.[4] Two other patients were reported to have the same compound heterozygous *LPL* variants as in our patient in the literature. One presented at 28 years of age with acute pancreatitis during late pregnancy, which is physiologically associated with hypertriglyceridaemia, and the other was diagnosed at 58 years of age after three episodes of pancreatitis.[5]

The *LPL* Ala98Thr and Leu279Val variants have allele frequencies of 33/19950 (0.17%) and 31/19954 (0.16%) among East Asians in the Genome Aggregation Database v2.1.1. Because the variants are unlinked,[4,5] this suggests a much higher prevalence of lipoprotein lipase deficiency among East Asians at around 1 in 100,000, which is still an underestimate as other disease-causing variants have not been taken into account. Widespread implementation of genetic testing should help uncover more undiagnosed cases among East Asians.

Surprisingly, our now 78-year-old patient has never suffered from pancreatitis or recurrent abdominal pain. This is unusual as most patients (50−80%) with lipoprotein lipase deficiency will develop pancreatitis.[1] Our patient had good past health until 56 years of age when hypertriglyceridaemia was discovered after an episode of transient ischaemic attack.

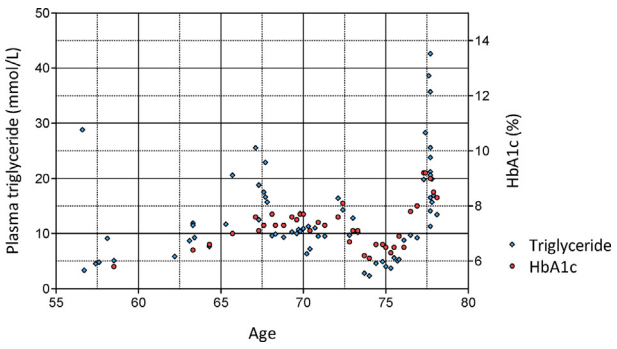

Fig. 1 Plasma triglyceride (mmol/L; left axis) and HbA1c (%; right axis) plotted against age of our patient.