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CORRESPONDENCE

Recommendations for the reporting of B cell populations in the context of common variable immunodeficiency disorder (CVID)

Sir,

The enumeration of B cell subsets by flow cytometry is increasingly being performed as a routine diagnostic test in laboratories, both in Australasia and internationally. Results of pilot External Quality Assurance (EQA) programs performed by The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) have shown that at least 16 Australasian pathology laboratories are routinely reporting B cell subset panels. These pilot studies have also revealed a marked discrepancy in the panels (number and type of fluorochromes used), as well as the reporting algorithm (if cell populations are reported as % of total lymphocytes or % of B cells). This has led to significant variation in the values of B cell subsets reported. In response to this, a review was undertaken to determine the minimum standards that laboratories should adhere to when reporting B cell subsets by flow cytometric methods.

The reduction or absence of B cell populations have been linked to a range of immunodeficiency syndromes including common variable immunodeficiency disorder (CVID). CVID is a complex disease that predisposes patients to recurrent and severe infections.[1–6] Diagnosis can be difficult and may be delayed due to the complex clinical phenotypes, that in addition to infections, may include lymphoproliferative, granulomatous and/or autoimmune manifestations.[1] B cell phenotyping may help to identify and classify patients earlier, allowing for timely and appropriate therapy and clinical interventions,[1–6] minimising the significant incidence of clinical comorbidities and complications often seen in CVID.[2,4]

Currently the diagnosis of CVID is based on the European Society for Immunodeficiency (ESID) diagnostic criteria of CVID. These criteria require defined clinical presentations, together with a marked decrease of IgG and IgA with or without low IgM levels (measured at least twice; with results at least 2 standard deviations lower than normal age appropriate levels) plus laboratory tests confirming either poor antibody response to vaccines and/or low switched memory B cells (<70% of age-related normal value). In addition to this, secondary causes of hypogammaglobulinaemia must be excluded, and the diagnosis must be established after 4 years of age with no evidence of profound T cell deficiency.[7]

Three seminal papers have proposed phenotypic and prognostic classification criteria for CVID patients based on B cell subpopulations. These are known as the 'Freiburg',[2] 'Paris'[3] and 'Euroclass'[4] classifications. Each classification has methodological variations, which make the interpretation of results in routine clinical practice difficult. To improve the standardisation of results reported by Australasian laboratories, we undertook a review of the classifications to establish minimum reporting requirements for diagnostic pathology laboratories who report B cell subset results used in the clinical characterisation of CVID (Table 1).

Based on this analysis, the following minimum recommendations are advised to provide clinically relevant information in the reporting of B cell subsets.

Recommendation 1: Sample type

Samples should be collected in ethylenediaminetetraacetic acid (EDTA).[2–5] The flow cytometric enumeration of B cell populations has been validated using both fresh peripheral blood and isolated PBMC methods, with good agreement.[5] B cell subsets should not be performed in the context of CVID when the total B cell count is <1% of peripheral blood lymphocytes (PBL).[2–5]

Recommendation 2: Populations and units reported

To provide clinically useful information for the classification of CVID, at a minimum, the B cell populations shown in Table 2 should be reported.

Recommendation 3: Reference ranges and interpretive commentary

The correct clinical interpretation of laboratory results is an essential outcome of laboratory services.[8] Quantitative data

Table 1 Analysis of Frieburg,[2] Paris[3] and EUROclass[4] classifications of B cell subsets, showing methodologies used and subpopulations reported in the analysis of CVID subgroups

	Freiburg	Paris	Euroclass
Sample used	EDTA PBMC	PBMC	EDTA whole blood or isolated lymphocytes
CVID patient group	CVID patients with B-cell counts >1% of PBLs and without evidence of granulomatous disease	CVID diagnosis according to IUIS criteria; all patients were adults with B cell counts >1% of PBLs	Patients >6 years of age, without evidence of malignancies, not on immunosuppressive treatment; B-cell counts >1% of PBLs
Naïve B cell ($CD19^+IgD^+$ IgM^+CD27^-) reported	Yes	Yes	Measured but not used in classification
Marginal zone/non-switched memory B cell ($CD19^+IgD^+$ IgM^+CD27^+) reported	Yes	Yes	Measured but not used in classification
Transitional B cells ($CD19^+CD38^{++}IgM^{high}$)	No	No	Yes
Switched memory B cell ($CD19^+IgD^-IgM^-CD27^+$) reported	Yes	Yes	Yes
CD 21 low ($CD19^+CD38^{low}CD21^{low}$) reported	Yes, reported as % of B cells	No, $CD21^+$ not $CD21^{low}$ used in analysis	Yes, reported as % of B cells
Plasmablasts ($CD19^+CD38^{+++}IgM^-$) reported	No	No	Measured but not used in classification

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Table 2 Minimum recommendation of phenotype and units reported in laboratories performing B cell subsets

Subset	Phenotype	Unit(s) reported
Total B cell	$CD19^+$	% of PBL
Naïve B cell	$CD19^+IgD^+$ IgM^+CD27^-	% of PBL and B cells
Marginal zone (non-switched memory B cell)	$CD19^+IgD^+$ IgM^+CD27^+	% of PBL and B cells
Transitional B cell	$CD19^+CD38^{++}IgM^{high}$	% of PBL and B cells
Switched memory B cell	$CD19^+IgD^-IgM^-CD27^+$	% of PBL and B cells
$CD21^{low}$	$CD19^+CD38^{low}CD21^{low}$	% of B cells

should be reported in the context of age appropriate established biological reference intervals and optimally, reported with an appropriate clinical interpretive comment. This is especially important in the context of treatment [access to intravenous immunoglobulins (IVIg) in Australia], which requires patients with CVID to meet the ESID criteria, which includes B cell phenotype.[8,9]

A significant amount of literature now exists on age-specific biological reference intervals, including paediatric populations.[2,3,10–12] Several studies have demonstrated the age dependent dynamic of B cell numbers and composition in peripheral blood, especially in children.[10,11] Adults have been shown to maintain relatively stable numbers of both immature/transitional and naïve B cells, whereas switched memory B cells and newly generated plasma cell numbers gradually decrease in subjects older than 60 years.[12] Reference intervals used by the laboratory should be documented as outlined in ISO 15189: Medical laboratories - Requirements for quality and competence.

These recommendations aim to standardise the reporting of B cell subset enumeration in routine clinical practice to increase the clinical utility of this assay. These recommendations are the minimum requirements that Australasian laboratories should follow when performing B cell subsets.

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Louise Wienholt[1], Michael Lane[2,3], Alice Grey[4], Tiffany Hughes[5]

[1]The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP), St Leonards, Sydney, NSW, Australia; [2]Division of Immunology, Pathology Queensland, Brisbane, Qld, Australia; [3]Faculty of Medicine, University of Queensland, Brisbane, Qld, Australia; [4]Department of Immunology, Royal Prince Alfred Hospital, Sydney, NSW, Australia; [5]Clinical Immunology and Allergy Department, The Royal Adelaide Hospital, Adelaide, SA, Australia

Contact Louise Wienholt.
E-mail: louise.wienholt@rcpaqap.com.au

1. Berglund LJ, Wong SWJ, Fulcher DA. B-cell maturation defects in common variable immunodeficiency and association with clinical features. *Pathology* 2008; 40: 288–94.
2. Warnatz K, Denz A, Drager R, *et al*. Severe deficiency of switched memory B cells (CD27(+)IgM(−)IgD(−)) in subgroups of patients with common variable immunodeficiency: a new approach to classify a heterogeneous disease. *Blood* 2002; 99: 1544–51.
3. Piqueras B, Lavenu-Bombled C, Galicier L, *et al*. Common variable immunodeficiency patient classification based on impaired B cell memory differentiation correlates with clinical aspects. *J Clin Immunol* 2003; 23: 385–400.
4. Wehr C, Kivioja T, Schmitt C, *et al*. The EUROclass trial: defining subgroups in common variable immunodeficiency. *Blood* 2008; 111: 77–85.
5. Ferry BL, Jones J, Bateman EA, *et al*. Measurement of peripheral B cell subpopulations in common variable immunodeficiency (CVID) using a whole blood method. *Clin Exp Immunol* 2015; 140: 532–9.
6. Bonilla FA, Barlan I, Chapel H, *et al*. International Consensus Document (ICON): common variable immunodeficiency disorders. *J Allergy Clin Immunol Pract* 2016; 4: 38–59.
7. European Society for Immunodeficiencies (ESID). *Diagnostic criteria of CVID*. Cited 5 Apr 2019. https://esid.org/Working-Parties/Registry/
8. Plebani M. The clinical importance of laboratory reasoning. *Clin Chim Acta* 1999; 280: 35–45.
9. National Blood Authority Australia. *Criteria for the clinical use of intravenous immunoglobulin in Australia*. Cited 10 Jan 2019. https://www.blood.gov.au/ivig-criteria
10. Morbach H, Eichhorn EM, Liese J, *et al*. Reference values for B cell subpopulations from infancy to adulthood: age-dependent reference values for B cell populations. *Clin Exp Immunol* 2010; 162: 271–9.
11. Piątosa B, Wolska-Kuśnierz B, Pac M, *et al*. B cell subsets in healthy children: reference values for evaluation of B cell maturation process in peripheral blood. *Cytometry B Clin Cytom* 2010; 78: 372–81.
12. Blanco E, Pérez-Andrés M, Arriba-Méndez S, *et al*. Age-associated distribution of normal B-cell and plasma cell subsets in peripheral blood. *J Allergy Clin Immunol* 2018; 141: 2208–19. e16.

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Simultaneous cytomegalovirus glomerulitis and BK virus nephropathy leading to kidney allograft loss

Sir,
Viral reactivations are a major cause of morbidity and mortality in immunocompromised patients. In kidney transplant recipients, BK polyomavirus is one of the most challenging opportunistic pathogens due to high prevalence, frequent reactivation and poor graft outcome. BK virus belongs to the family of Polyomaviridae and has a tropism for the renourinary tract which represents a site of viral latency. BK virus nephropathy (BKN) is usually encountered in a context of over-immunosuppression and affects up to 10% of kidney transplant recipients, resulting in 60% graft loss.[1] Kidney allograft biopsy remains the gold standard for BKN diagnosis. By contrast, CMV (cytomegalovirus) nephropathy is rare, detected in <1% of biopsied renal allografts,[2] but CMV infection or disease is an independent risk factor for patient death and long-term allograft dysfunction. Although BK and CMV serological reactivations may be associated in the kidney transplantation context,[3–5] histologically-proven co-infection in the kidney allograft is an exceptional finding. We report a case of simultaneous CMV glomerulitis and BKN leading to kidney allograft loss.

A 68-year-old woman with end-stage renal disease secondary to unknown glomerulopathy received a kidney from a cadaveric donor, with graft function (serum creatinine level 81 µmol/L) immediately after transplantation. CMV status was donor-positive (D+)/recipient-positive (R+). The patient received basiliximab, tacrolimus, mycophenolate mofetil and methylprednisolone for induction, followed by maintenance immunosuppression with tacrolimus, mycophenolate mofetil,