## Appendix 4: Specialist immunological investigations (immunology services only)

Specialist immunological tests are required in a small proportion of patients with bronchiectasis to make a diagnosis of primary immune deficiency, classify distinctive disease variants or to stage the extent of immune deficiency. Detailed investigation of the immune system should be undertaken by diagnostic laboratories which are externally accredited by appropriate regulatory bodies using validated standardized laboratory tests and a definitive diagnosis of immune deficiency should be based on established and accepted criteria[1-3]. Clinicians should be aware that some laboratory tests of immune function are not quality assured and that definitive guidelines of diagnosis of primary immune deficiencies may be modified as our understanding of the immune system increases.

### Enumeration of T and B cell counts in the diagnosis of primary and secondary antibody deficiency syndrome

Analysis of B and T cell counts plays an important role in diagnosis of a number of primary and secondary immune deficiencies which are associated with bronchiectasis. Reduced B cell counts can be seen in patients with CVID [3], reduced or absent B cell numbers are characteristic features of thymoma and immune deficiency [4], and also occur in 45% of patients with rituximab induced symptomatic antibody deficiency [5 6]. There is evidence that some patients with idiopathic bronchiectasis may have reduced B cell counts, however clinical significance and implications for management await further studies [7 8]. Low CD4 T cell counts may be observed in patients with HIV-1 infection, CVID, and thymoma with immune deficiency [3 5].

### Analysis of naive and memory B and T cell proportions in classification of patient with primary immune deficiencies and selection of individual patients for further genetic testing

Measurement of class switched memory B cell and of naïve CD4 T cell percentages have been incorporated into recent ESID guidelines for the diagnosis of CVID [9]. A marked reduction in naïve CD4 T cell proportion (less than 10%) in conjunction with low CD4 T cell counts (less than 200 cells/ul) in patients with a history of opportunistic T cell associated infections or significant granulomatous disease should raise the possibility of late onset combined immunodeficiency (LOCID) [10]. Identification of this patient group should prompt further investigations using next generation sequencing technology for gene mutations causing immune deficiency. In a large multi-centre French study of CVID patients the prevalence of bronchiectasis in patients with LOCID receiving IgG therapy was significantly higher than that observed in CVID patients which implies that a T cell defect may also be a contributory factor to development of progressive lung disease. Assessment of memory B cell proportions has been used to classify patients with CVID into homogeneous disease groups and potentially select individual patients for further genetic testing [11]. Reduced memory B cell subsets have been linked to increased rates of bronchiectasis in CVID patients [8 12-15] and poorer pneumococcal serotype vaccine responses[16 17] . Expansion of terminally differentiated CD8 T cells in response to persistent EBV and/or CMV viraemia, reduced naïve CD4 T cell and memory CD27+ B cells are characteristic features of patients with gain of function in the gene encoding p110δ subunit of phosphatidylinositol-3-OH kinase (PI (3) K) who may potentially benefit from rapamycin or PI(3)K inhibitor therapy [18 19].

### Measurement of IgG subclasses

IgG subclass deficiency is associated with increased susceptibility to bacterial sino-pulmonary infection and bronchiectasis, but the clinical value of measuring IgG subclasses is controversial and it is not usually recommended in the initial investigation of patients with suspected antibody deficiency [2]. Available tests to measure IgG subclasses are not standardised [20]. Concentrations of IgG2 and IgG3 are influenced by genetic variation in composition of IgG heavy chains and steroid therapy [21 22]. Reduced levels of individual IgG subclasses are not uncommon in healthy controls and not necessarily associated with an increased risk of bacterial infection [21]. In addition low IgG subclasses may spontaneously recover which means that reduced IgG subclass values should be confirmed by two tests at least 1 month apart [2 21]. Antibody responses to polysaccharide are not exclusively restricted to IgG2 subclasses [21] and there are very limited data to suggest to suggest that correction of IgG subclass deficiency using intravenous or subcutaneous immunoglobulin replacement therapy results in a clinically meaningful reduction in bacterial chest infections [22 23].

The prevalence of deficiency in one or more IgG subclasses in patients with bronchiectasis ranges from 6 to 48% [1 7 24-27]. Discrepancy between studies is largely explained by differences in criteria used to define IgG subclass deficiency and inclusion of reduced IgG4 concentration which is not regarded as a marker of humoral immune deficiency [2]. The prevalence of IgG1, IgG2, or IgG3 deficiency in patients with bronchiectasis is less than 1% in studies using more stringent criteria to define this condition [24 25 27].

The clinical significance of isolated reduction in IgG1, IgG2 and IgG3 should be confirmed by Pneumovax II test immunisation in patients with low baseline pneumococcal antibody levels [2]. IgG subclass deficiency is associated with an increased risk of sino-pulmonary infection in patients with selective IgA deficiency (IgA concentration less than 0.07g/L) and measurement of IgG subclasses should be considered in patients with co-existing defects in pneumococcal vaccine responses and composition of memory B cell subsets [28-30]. Measurement of IgG subclasses should also be considered in patients with a clinical suspicion of activated PI(3)Kδ (APDS) syndrome [19 20]. Assessment of IgG subclasses may also be considered in patients with impaired pneumococcal vaccine responses to distinguish between specific polysaccharide antibody and IgG subclass deficiency even though principles of management for both disorders are very similar [2].

### Assessment of MBL genotype and function

Mannose binding lectin (MBL) is an innate immune protein which recognizes cell surface carbohydrates expressed by a wide variety of bacteria, viruses and fungi [31]. MBL activates the lectin complement pathway resulting in opsonisation and phagocytosis of microorganisms and may also contribute to resolution of inflammation in the lung by increased elimination of apoptotic cells and suppression of inflammatory immune responses. Genetic polymorphisms in the MBL exon and promoter region influence MBL concentration and function. MBL deficiency is common (10-30% of population depending on the definition used) [32] but as yet there is no evidence that MBL deficiency increases susceptibility to infection [32] although it may modify disease course in patients with co-existing inherited or acquired immune deficiencies [31]. For example there is a higher rate of bronchiectasis in CVID if MBL deficiency is also present [33 34], and meta-analysis of 12 studies of 2863 adult patients with cystic fibrosis showed an association between low expressing MBL genotypes and earlier age of onset of Pseudomonas aeruginosa infection, chronic Burkholderia cepacia colonisation, impaired lung function, need for lung transplantation and/or mortality [35].

Only 2 studies have examined the relationship between MBL deficiency and severity of lung disease in non-CF bronchiectasis. McFarlane and colleagues showed in a retrospective cohort that there was no association between reduced serum MBL concentrations and severity of lung disease [36]. In contrast a larger prospective study showed low expressing MBL genotypes were associated with increased bacterial colonisation, infective exacerbations and hospital admissions, more severe CT scores, worse quality of life and increased airway inflammation compared to patients with intermediate and high expressing MBL genotypes [34]. Confirmation of these findings in further studies and evidence that improvement in MBL function influences clinical outcomes are needed before this test can be recommended in routine investigation of patients with bronchiectasis.

### Assessment of Haemophilus influenzae Type B (HIB) and tetanus toxoid antibodies

Measurement of serum levels of antigen-specific IgG antibody to vaccine antigens or documented infection is essential for diagnosis of primary antibody deficiency [1]. Evaluation of functional antibody responses should include measurement of antibody levels to T cell dependent protein or glycoprotein antigens and T cell independent polysaccharide antigens [1 9]. In clinical practice analysis of T cell dependent antibody function is usually assessed by analysis of tetanus toxoid antibodies (protein antigen) or *Haemophilus influenzae* Type B (HIB) polysaccharide capsule antigen coupled to a carrier protein (conjugate vaccine). Protective antibody levels to tetanus toxoid (0.15IU/mL) and to HIB polysaccharide capsule (1ug/mL) have been established [3 37 38]. Antibody levels below protective thresholds following test immunisation are believed to indicate impaired vaccine responses and a functional antibody deficiency syndrome.

A number of studies have examined the clinical utility of measurement of baseline and post vaccination HIB antibody levels in the diagnosis of immune deficiency in patients with bronchiectasis [25 26 39 40]. Reduced HIB vaccine responses

were reported by a Spanish group in patients with idiopathic bronchiectasis [26 39]. In contrast two UK studies showed that almost all patients with absent HIB antibodies mounted protective antibody responses post-test immunisation and concluded that measurement of HIB antibodies were not essential in the initial diagnostic investigation of patients with bronchiectasis[25 40]. Although differences in study setting, patient cohorts and HIB assay methods account in part for the discrepant findings reported, the most likely explanation is that the Spanish group compared vaccine responses to those observed in healthy controls whereas the UK groups analysed the proportion of patients who achieved protective antibody levels of 1ug/ml.

Absent protective antibody in UK adults may therefore reflect lack of exposure to HIB infection rather than a marker of susceptibility to infection. Finally the biological and clinical relevance of measurement of HIB antibodies is unclear given that the majority of *Haemophilus influenzae* infections in patients with bronchiectasis are caused by mucosal non typeable unencapsulated species [41]. Hence the use of HIB antibody measurements is unlikely to be clinically useful in assessment of patients with bronchiectasis and should be restricted to patient groups at increased risk of systemic HIB infection (major antibody syndromes such as suspected CVID and/or XLA).

Measurement of tetanus toxoid antibody levels can also be used to assess T cell dependent antibody function. Protective levels to tetanus toxoid have been defined and most adults receiving a single booster dose of tetanus toxoid should achieve an antibody response of at least 1IU/mL [42]. Routine measurement of tetanus antibodies is of limited value in individuals over the age of 55-60 since administration of tetanus toxoid was only incorporated into standard UK childhood immunisation programme in 1961. It is important to clarify if individuals in this age group have ever received tetanus vaccine to see if measurement of tetanus antibodies and test immunisation is indicated. Some antibody deficiency patients may have pre-existing protective tetanus antibody levels and can make protective tetanus antibody levels post vaccination due to generation of vaccine specific memory B cells prior to onset of humoral immune deficiency. One reason is that tetanus vaccine specific memory generated before onset of humoral immune deficiency may persist and function despite significant antibody deficiency. Given the uncertainty about definition of normal and/or protective post immunisation pneumococcal levels, assessment of tetanus antibody and vaccination in those with non-protective and/or low levels (less than 0.5IU/mL) should be considered in patients in whom IgG replacement is being considered.

## Testing for molecular defects

Whole exome and more recently whole genome sequencing have identified a number of primary antibody deficiencies associated with bronchiectasis [18 19]. Although these techniques are predominantly research based, continuing advances in automated DNA sequencing technology, bio-informatic analysis, data handling and storage and efforts to educate healthcare personnel mean that genomics will be an important part of specialist investigations within the next few years. Identification of genetic defects may be desirable in patients presenting with familial history of primary antibody deficiency, consanguinity, bronchiectasis associated with herpes viral infection and lymphoma. Results of immunological investigation may also guide further testing for molecular defect, absent B cells, or marked reduction in class switched memory B cells in patients with CVID [11 43] or reduced IgG and IgA and normal/high IgM and alteration in abnormal T cell immune profiles deficiency [18 19]. Identification of biological pathways predisposing to respiratory tract infections using next generation DNA sequencing has already led to discovery of novel causes and potential treatment for patients with antibody deficiency and bronchiectasis [44 45]. It is hoped that finding derived from inborn errors of immunity may also result in novel therapeutic approaches for patients with idiopathic bronchiectasis.


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