Published in final edited form as:
J Med Genet. 2015 December ; 52(12): 856–859. doi:10.1136/jmedgenet-2015-103266.

Cowden's syndrome with immunodeficiency

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Abstract

Background—Cowden's syndrome is a rare, autosomal dominant disease, caused by mutations in the phosphoinositide 3-kinase and phosphatase and tensin homolog (*PTEN*) gene. It is associated with hamartomatous polyposis of the gastrointestinal tract, mucocutaneous lesions, and increased risk of developing certain types of cancer. In addition to increased risk of tumour development, mutations in *PTEN* have also been associated with autoimmunity in both mice and humans. To date, however, an association between Cowden's syndrome and immune deficiency has been reported in a single patient only.

Methods and Results—Two patients with Cowden's syndrome and an increased frequency of infections were investigated for possible underlying immunodeficiency. In one patient, hypogammaglobulinaemia with a functional antibody deficiency was identified, whilst the other patient had a persisting CD4+ T cell lymphopenia (with normal antibody production).

Conclusions—Our data indicate that Cowden's syndrome may be associated with both T cell and B cell immune dysfunction. We recommend that patients with Cowden's syndrome and an increased frequency of infections are investigated for associated immunodeficiency.

Keywords

Cowden's syndrome; PTEN; immunodeficiency; antibody deficiency

Introduction

Cowden's syndrome is a rare, autosomal dominant disease, caused by mutations in the phosphoinositide 3-kinase and phosphatase and tensin homolog (*PTEN*) gene. It is associated with hamartomatous polyposis of the gastrointestinal tract, mucocutaneous lesions, and increased risk of developing certain types of cancer [OMIM #158350]. In this context, PTEN acts as a tumour suppressor (reported as being the second most commonly mutated tumour suppressor gene in sporadic human cancers [1]). PTEN is a phosphatase,

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which catalyzes the conversion of phosphatidylinositol(3,4,5)P_3 (PIP_3) to phosphatidylinositol(4,5)P_2, antagonizing the signalling cascades downstream of receptor tyrosine kinases (RTKs) and phosphatidylinositol-3-kinase (PI3K) [2]. Amongst its diverse roles, PI3K activation is involved in the development, activation and differentiation of both T and B lymphocytes [3]. Immune dysregulation, including impaired lymphocyte activation-induced apoptosis, lymphoid hyperplasia, and increased autoimmunity due to defects in T and B cell homeostasis, has been described in mice with targeted heterozygous *PTEN* mutations [4]. A recent report [5] highlighted a range of autoimmune manifestations and lymphoid hyperplasia in a series of patients with germline *PTEN* mutations. In addition, mice with a B cell-specific mutation in *PTEN* were shown to have reduced levels of IgG and IgA, impaired specific antibody production, and defective immunoglobulin class switch recombination (CSR) [6]. To date, however, there have been no reports of antibody deficiency in patients with Cowden's syndrome, and T cell deficiency has been reported in a single patient only [7].

Case Reports

We report the cases of two young male patients with Cowden's syndrome, associated with mutations in the *PTEN* gene, in whom investigation of frequent infections led to the identification of distinct underlying immune abnormalities.

Case 1

The first case is a 5 year old boy. He was noted to have macrocephaly antenatally, although no cause was identified initially. He was the only child of non-consanguineous parents. It was noted that his father also had macrocephaly. He was referred for a Genetics opinion at 15 months due to increased head circumference. In addition to macrocephaly, with an OFC >99.6th centile, there was evidence of mild developmental delay, but no other obvious features. Genetic testing confirmed the presence of a heterozygous mutation in the *PTEN* gene (*c.203A>G*). This mutation, in a highly conserved area in exon 3, encodes an amino acid substitution from tyrosine to cysteine (p.Tyr68Cys) within the phosphatase domain (Figure 1A). A tyrosine to histidine substitution at this position in PTEN has previously been described as abolishing PTEN phosphatase activity [8]. The same mutation was identified in his father. He had suffered from frequent respiratory tract infections from a young age, and had had 3 hospital admissions by the age of 4 years. Immunological investigations at 23 months showed a panhypogammaglobulinaemia (Table 1), with protective levels of specific antibody to tetanus toxoid, but non-protective specific antibodies to *H. influenzae type B* (Hib), in spite of prior immunization according to the UK vaccination schedule. He was started on prophylactic antibiotics, with a significant improvement in the frequency and severity of respiratory tract infections. In addition, he received a booster dose of the Menitorix vaccine, and a single dose of the unconjugated pneumococcal vaccine, Pneumovax II, to both of which he made good specific antibody responses (Table 1 and Supplementary Table). However, approximately 15 months later, his specific antibodies to Hib and pneumococcus had both fallen to non-protective levels, and his panhypogammaglobulinaemia persisted. His peripheral blood lymphocyte immunophenotypic profile showed normal or slightly elevated numbers of T cells, NK cells

and (polyclonal) B cells on two occasions, although he had increased proportions of both CD5+ and CD10+ B cells (Table 1). The results indicate a significant functional impairment of antibody production. Of note, he also had marked tonsillar hyperplasia.

Analysis of PI3K signalling was undertaken in isolated peripheral blood T cells from this patient. Western blot analysis showed a reduced level of PTEN protein expression (compared with a healthy control), with an increase in Akt and S6 phosphorylation following stimulation with anti-CD3 and anti-CD28, but an unexpected reduction in Foxo phosphorylation (Figure 1B). There was no difference in basal PIP_3 levels between the patient's T cells and those of the control (Figure 1C). Following activation, the patient's T cells showed slightly higher levels of PIP3 compared with the control, and these responses were completely inhibited by PI3K inhibitors CAL-101 and ZSTK474 (Figure 1C). The results suggest that the mutation had a relatively minor effect on PI3K signalling within the patient's T cells.

Case 2

The second case is a 10 year old boy who was diagnosed with Cowden's syndrome at approximately 3 years of age. Genetic analysis revealed a T to A substitution at base 87 (c. 87T>A) in one *PTEN* gene. This mutation encodes a STOP codon in place of a Tyrosine at amino acid position 29 in the PTEN protein (Figure 1A). His parents and a younger sister all tested negative for mutations in the *PTEN* gene. He had initially presented at 8 months of age, when he was also noted to have macrocephaly (head circumference above the 97th centile). He had suffered from recurrent acute ear and upper respiratory infections from an early age, and had had tympanostomy tubes (grommets) inserted on three occasions, adenoidectomy at 4 ½ years, and tonsillectomy at 8 ½ years. In spite of these interventions, he continued to suffer from recurrent episodes of otitis media. At age 9 years, in view of the ongoing infections, he had his serum immunoglobulin, specific antibody, and lymphocyte subset levels measured. Serum immunoglobulins were normal for age, and he had evidence of satisfactory antibody responses to tetanus toxoid and Hib vaccination in early childhood (Table 1). His specific antibody levels to pneumococcus (against which he had not been immunized) were low, and he was immunized with Pneumovax II, to which he made a normal antibody response (Table 1 and Supplementary Table). However, lymphocyte subset analysis showed a moderate CD4+ T cell lymphopenia and a mild CD8+ T lymphopenia, with normal numbers of NK cells and B cells. On re-testing 6 months later, the CD4+ T cell lymphopenia (both as % of lymphocytes and absolute numbers) persisted (Table 1).

There was no clinical or biochemical evidence of autoimmunity in either patient.

Discussion

We report 2 patients with heterozygous mutations in the *PTEN* gene in whom there was an increased frequency of (mainly respiratory tract) infections, in whom distinct immunological defects were identified. In addition to its function as a tumour suppressor, PTEN acts as an antagonist of PI3K activity in B and T cell differentiation and homeostasis [3,9,10]. Studies in mice deficient in PTEN have shown altered patterns of B cell differentiation, with expansion of B1 and marginal zone B cells, impaired CSR, and increased autoantibody

production [6,11-13], associated with sustained production of PIP3 [14]. Translating these studies to humans with germline *PTEN* mutations, Heindl et al [5] described an increased prevalence of lymphoid hyperplasia (gastrointestinal, tonsillar and thymic), and of autoimmune disease (Hashimoto's thyroiditis and autoimmune haemolytic anaemia). They reported no increase in susceptibility to infections, although total lymphocyte counts showed a tendency toward lower lymphocyte numbers [5]. Serum immunoglobulins were reported as being within "lower normal" range (although data presented showed reduced levels of IgG, IgA and IgM respectively in individual patients, in a group of 8 patients having serum immunoglobulins measured; specific antibody responses were not reported) [5]. The two patients reported in our study both presented with an increased frequency of infections in childhood, which prompted investigation of their immune functions. One of the patients showed a panhypogammaglobulinaemia. Specific antibody responses to immunization with the conjugate Hib vaccine, Menitorix, and the unconjugated pneumococcal polysaccharide vaccine, Pneumovax II, were initially normal (at 4 weeks post immunization), but the antibody levels declined rapidly, returning almost to baseline levels by 15 months post immunization, indicating a functional antibody deficiency. Major lymphocyte subpopulations were present in normal numbers in this patient, although there were increased proportions of CD5+ and CD10+ B cell subsets in the blood, a finding that has been noted previously in patients with *PTEN* mutations [5]. Our second patient had normal serum immunoglobulin levels, protective antibodies to tetanus and Hib, and a normal antibody response to pneumococcal polysaccharide antigen, but had a persistent CD4+ T cell lymphopenia. The two cases illustrate that abnormalities of T cell and B cell development and/or function may be present in patients with Cowden's syndrome, and may be associated with increased susceptibility to infections.

The difference in immunological phenotypes of our two patients, and their relation to patients with Cowden's syndrome more generally, are hard to explain. In both cases, the mutations would be predicted to abolish the phosphatase activity of the affected PTEN protein. Studies of PI3K function in patient 1 showed reduced expression of the PTEN protein and increased AKT and S6 phosphorylation (in peripheral blood T cells), but did not show clear differences in either basal or stimulated levels of PIP3 compared with a healthy control. The modest increase in PIP_s in T cells from our patient is consistent with the fact that patients with Cowden's syndrome have one allele of non-mutated *PTEN* which produces a functional protein, albeit it at reduced levels. Of interest, dominant gain-of-function mutations in *PIK3CD*, which encodes the p110δ subunit of PI3K, have recently been associated with a clinical syndrome involving antibody deficiency, CD4+ T cell lymphopenia, sinopulmonary infections and lymphoid hyperplasia, associated with increased phosphorylation of Akt kinase [15-17], emphasising the role of PI3K in lymphocyte homeostasis and function. In contrast to our patient with Cowden's syndrome, patients with the E1021K gain-of-function mutation of PI3Kδ showed a 2-3 fold increase in basal and stimulated PIP_3 levels compared with controls [15]. The effects of (heterozygous) *PTEN* mutations on lymphoid development and function would therefore appear to share some features with gain-of-function mutations in *PIK3CD*, but with a less severe clinical phenotype.

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Immunodeficiency has been reported previously in a single patient with Cowden's syndrome [7], who presented with recurrent cellulitis and abscesses, and reduced T cell numbers and in vitro T cell proliferative function. This patient had normal levels of serum immunoglobulins. Given the known role of PTEN in regulating PI3K activation, and the function of PI3K in the differentiation and homeostasis of both B and T cells, it is perhaps surprising that immune problems have not been reported more widely in patients with Cowden's syndrome. Based on our observations, we recommend measuring serum immunoglobulins, specific antibody levels and lymphocyte subpopulations in patients with Cowden's syndrome who show an increased susceptibility to infections.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We would like to thank Jonathan Clark, Babraham Institute, for help with lipid measurements. Work in KO's lab was funded by the BBSRC and GSK.

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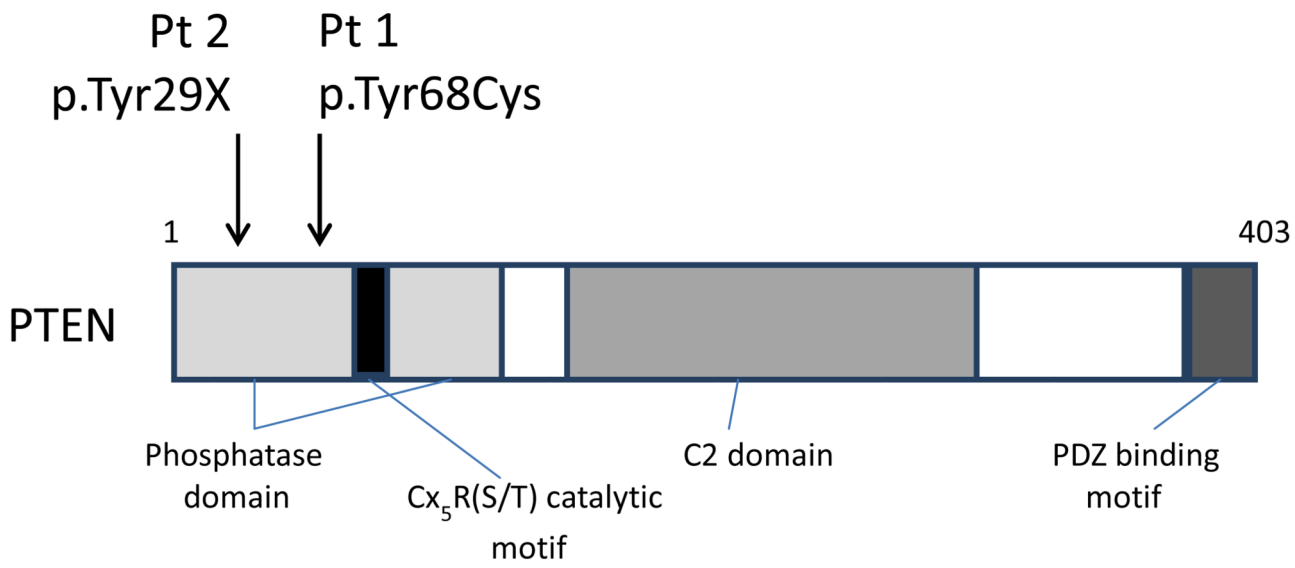
A)



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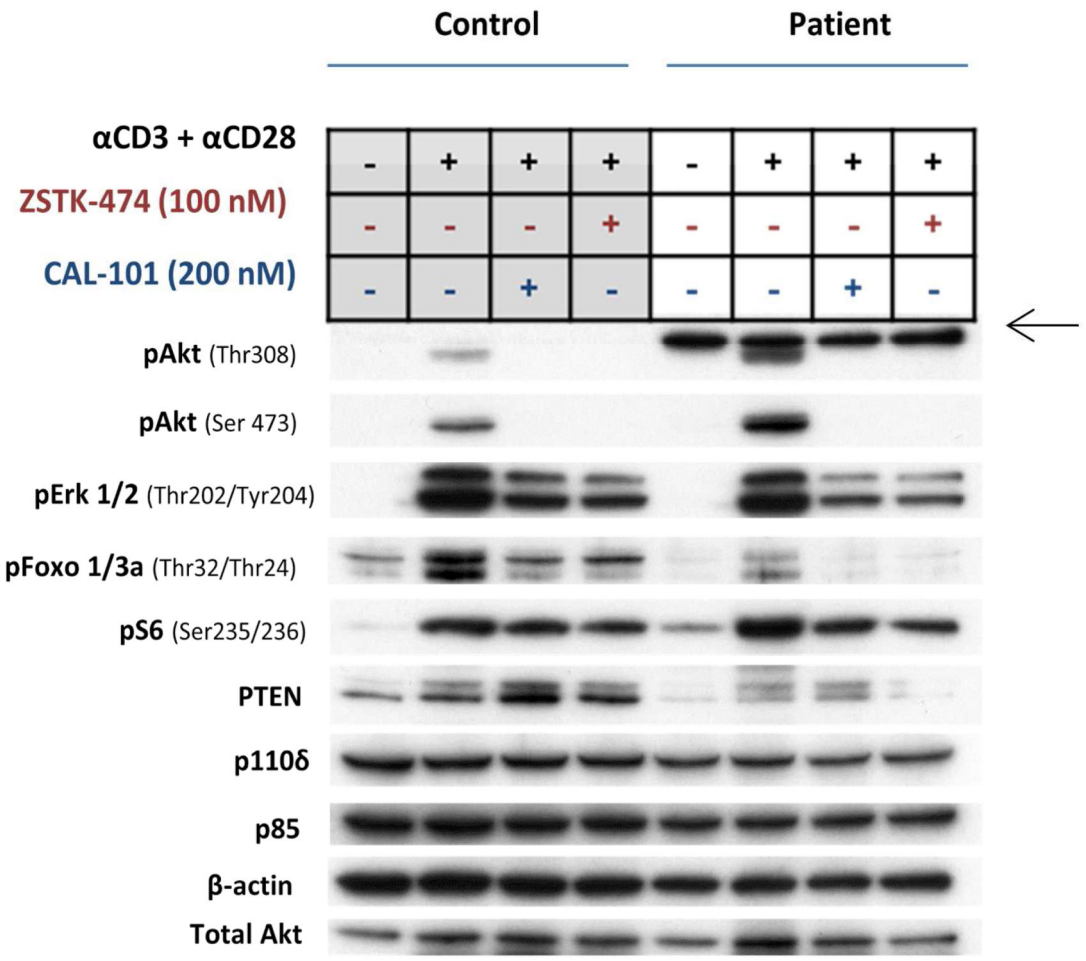

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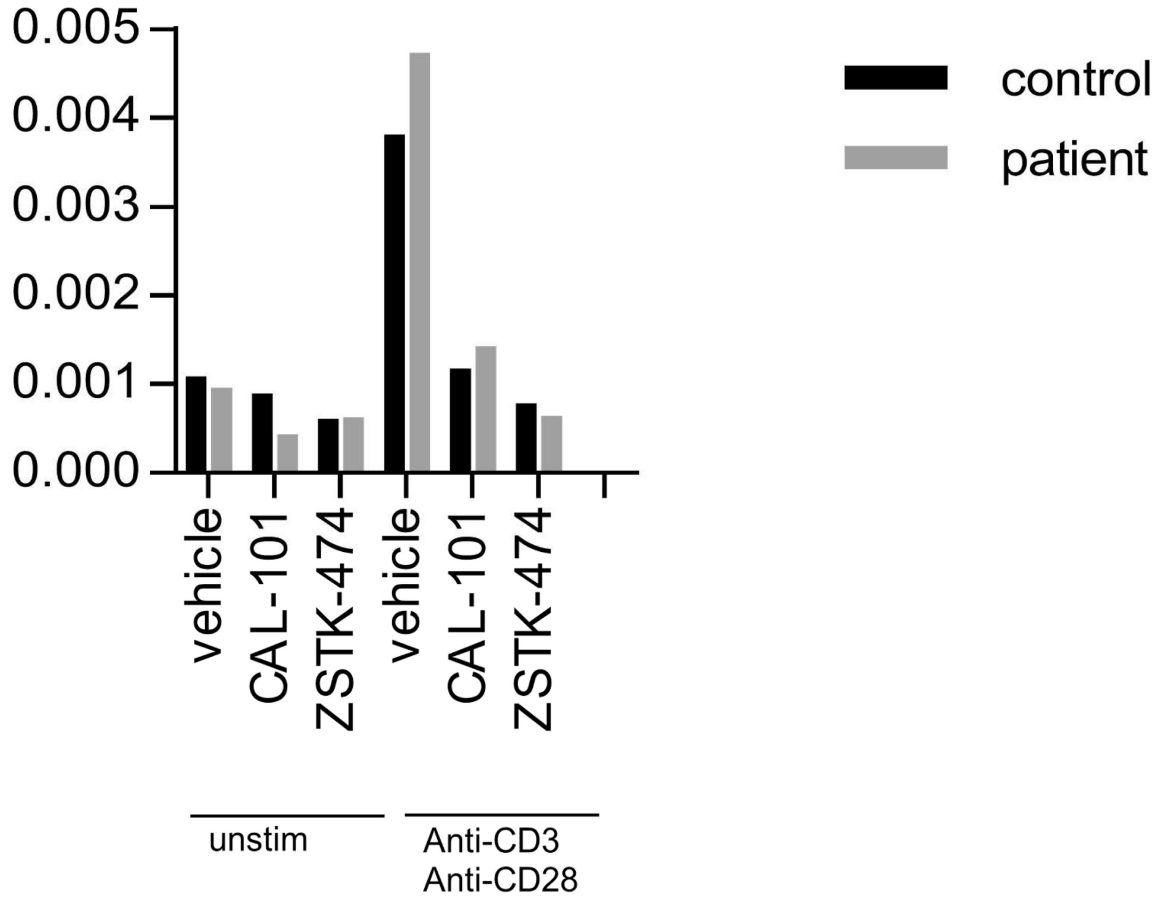

Figure 1.

A) Schematic representation of the PTEN protein, with locations of the mutations identified in Patients 1 and 2 indicated; B) Western blot analysis and C) PIP_3 levels in peripheral blood T cells from Patient 1, stimulated in vitro with anti-CD3 and anti-CD28 in the presence or absence of CAL-101 (Idelalisib; PI3Kδ inhibitor) or ZSTK474 (class I PI3K inhibitor). Western blotting and PIP_3 analysis were performed as described previously [15,18]. [A consistent, non-specific band with slightly high molecular weight (arrowed in Fig 1B), was seen in the patient's pAkt Thr308 western blot (but not in the control), which was not inhibited by ZSTK-474 or CAL-101].

Table 1

Immune parameters in Patients 1 and 2.

	Patient 1 (@ 23 months)[1]	Patient 1 (post-immunization)[2]	Patient 1 (@ 44 months)[3]	Patient 2 (@ 9 years 2 months)[1]	Patient 2 (post-immunization)[2]
IgG	**2.9** (3.1-13.8)	NT	**3.6** (4.9-16.1)	10.4 (5.4-16.1)	NT
IgA	**0.14** (0.3-1.2)	NT	**0.33** (0.4-2.0)	0.86 (0.7-2.5)	NT
IgM	**0.37** (0.5-2.2)	NT	**0.40** (0.5-2.2)	1.2 (0.5-1.8)	NT
Tetanus toxoid [4]	0.18	0.93	0.33	0.39	NT
H. influenzae B [4]	0.44	8.71	0.45	0.92	NT
Pneumococcus (% serotypes >0.35 mg/L)[5,6]	NT	100%	54%	8%	100%
Absolute lymphocyte count	NT	8.03 (2.3-5.4)	**2.19** (2.3-5.4)	**1.11** (1.9-3.7)	**1.36** (1.9-3.7)
CD3+ T cells (%)	NT	62.6 (56-75)	71.3 (56-75)	63.4 (60-76)	66.2 (60-76)
CD3+ T cells (abs)	NT	5.03 (1.4-3.7)	1.56 (1.4-3.7)	**0.70** (1.2-2.6)	**0.90** (1.2-2.6)
CD4+ T cells (%)	NT	31.8 (28-47)	42.4 (28-47)	**27.6** (31-47)	**26.4** (31-47)
CD4+ T cells (abs)	NT	2.55 (0.7-2.2)	0.93 (0.7-2.2)	**0.31** (0.65-1.5)	**0.36** (0.65-1.5)
CD8+ T cells (%)	NT	23.1 (16-30)	22.9 (16-30)	29 (18-35)	31.4 (18-35)
CD8+ T cells (abs)	NT	1.85 (0.49-1.3)	0.50 (0.49-1.3)	**0.32** (0.37-1.1)	0.43 (0.37-1.1)
CD16/56+ NK cells (%)	NT	7.7 (4-17)	5.4 (4-17)	9.4 (4-17)	13.1 (4-17)
CD16/56+ NK cells (abs)	NT	0.62 (0.13-0.72)	**0.12** (0.13-0.72)	0.10 (0.1-0.48)	0.18 (0.1-0.48)
CD19+ B cells (%)	NT	28.9 (14-33)	22.9 (14-33)	25.9 (13-27)	20.4 (13-27)
CD19+ B cells (abs)	NT	2.32 (0.39-1.4)	0.50 (0.39-1.4)	0.29 (0.27-0.86)	0.28 (0.27-0.86)
CD19+/CD5+ B cells (%)	NT	NT	*46.8*	2.3	6.1
CD19+/CD10+ B cells (%)	NT	NT	*16.1*	2.6	1.7

Serum immunoglobulins are reported in g/L, tetanus toxoid antibodies in IU/ml, and Hib and pneumococcal antibodies in mg/L. Absolute lymphocyte subset counts are reported as number of cells $\times 10^9$/L. Age related normal ranges, where available, are given in parenthesis below the patient's values. Abnormally low values (for serum immunoglobulins and lymphocyte subsets) are presented in bold type.

[1] Pre-immunization;

[2] 1 month post-immunization with Menitorix and Pneumovax II;

[3] 15 months post-immunization;

[4] Protective level for tetanus toxoid = >0.15 IU/ml; protective level for Hib = >1.0 mg/L;

[5] Putative protective serotype specific pneumococcal antibody level = 0.35 mg/L;

[6] Full serotype specific pneumococcal antibody levels are given in the Supplementary Table; NT = not tested.