Mining the antibiogram: what more can it tell us?

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The subtyping of bacterial isolates is important for longitudinal epidemiological investigations, as well as for acute outbreak analysis, in order to help guide clinical management of such outbreaks. Although several molecular techniques, including pulsed-field gel electrophoresis (PFGE)[1] and DNA sequence typing,[2] have been described in the literature, relatively few clinical microbiology laboratories currently employ such techniques routinely, and may refer isolates to specialist laboratories or reference laboratories for subsequent analysis. Instead, when faced with such a typing dilemma, many routine clinical microbiology laboratories will revert to in-house methods of discrimination at their disposal to assist with preliminary investigations until more data are returned from specialist laboratories employing more discriminatory subspecies typing techniques.

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As a result, in such circumstances, many laboratories will examine the antibiogram information relating to a set of isolates which are believed to be related. To date, this analysis has been performed qualitatively without the aid of any statistical software, whereby the clustering of isolates into specific 'resistotypes' has been done manually. This method has worked adequately but becomes more complicated when large numbers of isolates are involved, as well as when extended antibiograms are employed.

Therefore, it is the aim of this study to evaluate the biostatistical software Biostatistica (SoftStat, Tulsa, USA) in the analysis of antibiograms to aid cluster analysis and information management.

The joining or tree clustering method uses similarities of antibiotic susceptibility when forming the clusters. In this particular case, similarities are the organisms' responses to a set of antibiotics tested under standardised conditions. The most straightforward way of computing distances between objects in a multidimensional space is to compute Euclidean distances. Euclidean distance is simply the geometric distance in the multidimensional space and is computed as distance(x,y) = $(\Sigma_i [x_i - y_i]^2)^{\frac{1}{2}}$.

Table 1. Antibiotic susceptibility of 10 hypothetical outbreak-related isolates and 11 sporadic isolates against six antibiotic agents (vancomycin, penicillin, gentamicin, ciprofloxacin, erythromycin, tetracycline).

	Antibiotic susceptibility					
Hypothetical isolate	**Vancomycin**	**Penicillin**	**Gentamicin**	**Ciprofloxacin**	**Erythromycin**	**Tetracycline**
Outbreak-related isolates						
Outbreak 1	R	R	S	S	R	R
Outbreak 2	R	R	S	S	R	R
Outbreak 3	R	R	S	S	R	R
Outbreak 4	R	R	S	S	R	R
Outbreak 5	R	R	S	S	R	R
Outbreak 6	R	R	S	S	R	R
Outbreak 7	R	R	S	S	R	R
Outbreak 8	R	R	S	S	R	R
Outbreak 9	R	R	S	S	R	R
Outbreak 10	R	S	S	R	S	S
Sporadic isolates						
Sporadic 1	R	S	S	R	S	S
Sporadic 2	R	S	S	S	S	S
Sporadic 3	R	R	S	R	S	R
Sporadic 4	R	S	S	S	S	R
Sporadic 5	R	R	S	R	S	S
Sporadic 6	R	R	S	S	S	S
Sporadic 7	R	S	S	R	S	S
Sporadic 8	R	S	S	S	S	R
Sporadic 9	R	S	S	R	S	R
Sporadic 10	R	S	S	S	S	R
Sporadic 11	R	S	S	R	S	R

S: susceptible, R: resistant.

As a working example, mathematical clustering analysis was computed employing Euclidean distances from existing antibiograms using the commercially available Biostatistica statistical software (SoftStat) on a hypothetical outbreak scenario.

An unusually high number of laboratory reports ($n=10$) of organism X were received suggesting the occurrence of an outbreak with organism X. The resulting antibiogram obtained by standard disk-diffusion antibiotic susceptibility testing of all six antibiotics (vancomycin, penicillin, gentamicin, ciprofloxacin, erythromycin, tetracycline) tested against the presumptive outbreak isolates is shown in Table 1, along with sporadic isolates preceding the query outbreak. Antibiotic susceptibility data were transposed into an unweighted matrix, where 1 represents sensitivity and 2 is non-susceptibility (intermediate and fully resistant; Table 2).

In addition to the hypothetical working example, as detailed above, three actual case studies were analysed in order to demonstrate this proof of principle. These were i) longitudinal analysis of antibiotic susceptibility of mucoid *Pseudomonas aeruginosa* isolated from the airways of an adult patient with cystic fibrosis (CF), ii) antibiotic susceptibility-relatedness of a population of viridans-group streptococci; and iii) analysis of a gastrointestinal outbreak of *Salmonella typhimurium*.

The antibiogram of mucoid *P. aeruginosa* was examined from eight isolates obtained from the sputum of an adult patient with CF during the period July 2008 to November 2009. Antibiotic susceptibility of all isolates had been tested using the same methodology against 11 antibiotics, including aminoglycosides (amikacin, gentamicin, tobramycin), β-lactams (aztreonam, ceftazidime, imipenem, meropenem, temocillin, tazobactam/piperacillin), fluoroquinolones (ciprofloxacin) and polymyxins (colistin). Antibiotic susceptibility was recorded as either susceptible (sensitive) or non-susceptibile (moderately or totally resistant).

The antibiogram of a population of viridans-group streptococci (VGS; $n=48$) obtained from nasal and oropharyngeal swabs from individuals was examined against seven antibiotics, including penicillin, oxacillin, erythromycin, norflaxacin, ciprofloxacin, levoflaxacin and moxifloxacin. In this case, susceptibility zones were measured (mm) and input into an unweighted matrix for Euclidean distance determination.

A family outbreak of gastrointestinal disease due to *S. typhimurium* involving five family members was analysed using an extended antibiogram of 12 antibiotics, including ampicillin, augmentin, chloramphenicol, ciprofloxacin, gentamicin, sulphonamide, tetracycline, trimethoprim, kanamycin, nalidixic acid, streptomycin and furazolidone. In addition, a further eight isolates of *S. typhimurium*, which had been isolated contemporaneously and not believed to have been involved in the family outbreak, were also examined.

The hypothetical susceptibilities to six antibiotics are shown in Table 1 for the 10 outbreak-related and 11 sporadic isolates. Table 2 shows the transposition of qualitative

Table 2. Conversion of antibiotic susceptibility data from qualitative to digital format.

	Unweighted matrix					
Hypothetical isolate	Vancomycin	Penicillin	Gentamicin	Ciprofloxacin	Erythromycin	Tetracycline
Outbreak-related isolates						
Outbreak 1	2	2	1	1	2	2
Outbreak 2	2	2	1	1	2	2
Outbreak 3	2	2	1	1	2	2
Outbreak 4	2	2	1	1	2	2
Outbreak 5	2	2	1	1	2	2
Outbreak 6	2	2	1	1	2	2
Outbreak 7	2	2	1	1	2	2
Outbreak 8	2	2	1	1	2	2
Outbreak 9	2	2	1	1	2	2
Outbreak 10	2	1	1	2	1	1
Sporadic isolates						
Sporadic 1	2	1	1	2	1	1
Sporadic 2	2	1	1	1	1	1
Sporadic 3	2	2	1	2	1	2
Sporadic 4	2	1	1	1	1	2
Sporadic 5	2	2	1	2	1	1
Sporadic 6	2	2	1	1	1	1
Sporadic 7	2	1	1	2	1	1
Sporadic 8	2	1	1	1	1	2
Sporadic 9	2	1	1	2	1	2
Sporadic 10	2	1	1	1	1	2
Sporadic 11	2	1	1	2	1	2

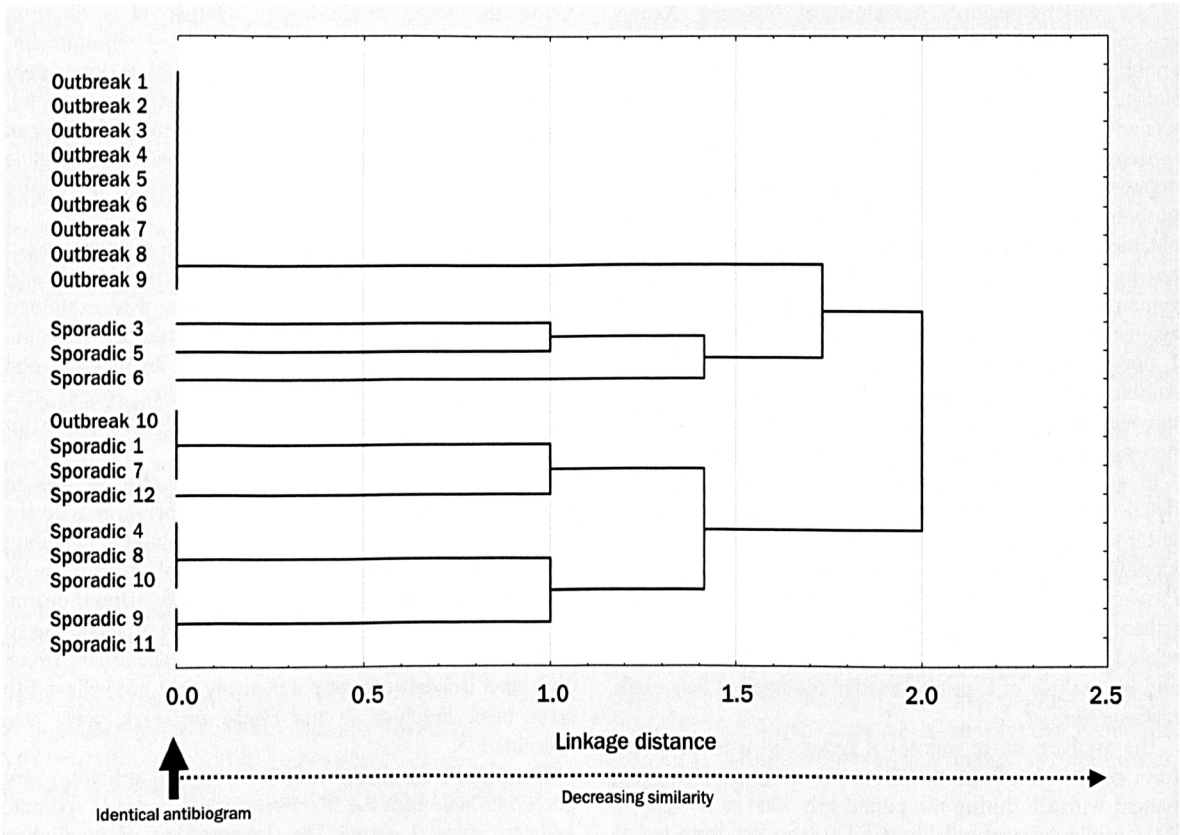

Fig. 1. Dendogram of isolate relatedness based on complete linkage of Euclidean distances among six antibiotic agents in a hypothetical/working example outbreak case scenario.

susceptibility data from Table 1 into quantitative (digital) data, which then generated a clustering matrix based on complete linkage, forming a dendogram of relatedness (Fig 1). The linkage distance value along the x axis is an arbitrary value of relatedness calculated by the statistical software. Identical antibiograms have a linkage distance of 0.0 and the linkage distances further from zero equate to decreasing relatedness. This Figure demonstrates that there are four clusters, each with identical antibiograms, as well as three sporadic isolates (sporadic isolates 3, 5 and 6). Outbreak

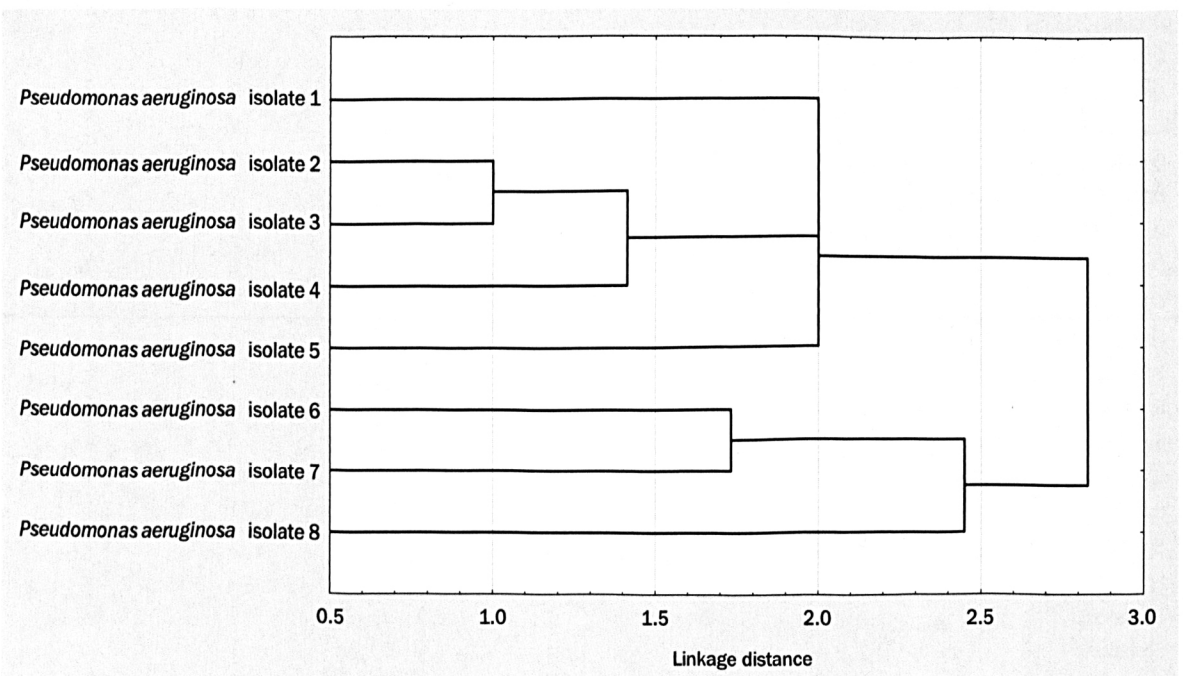

Fig. 2a. Dendogram of isolate relatedness based on complete linkage of Euclidean distances with 11 antibiotic agents examined with eight isolates of *Pseudomonas aeruginosa* from an adult CF patient over a 16-month period.

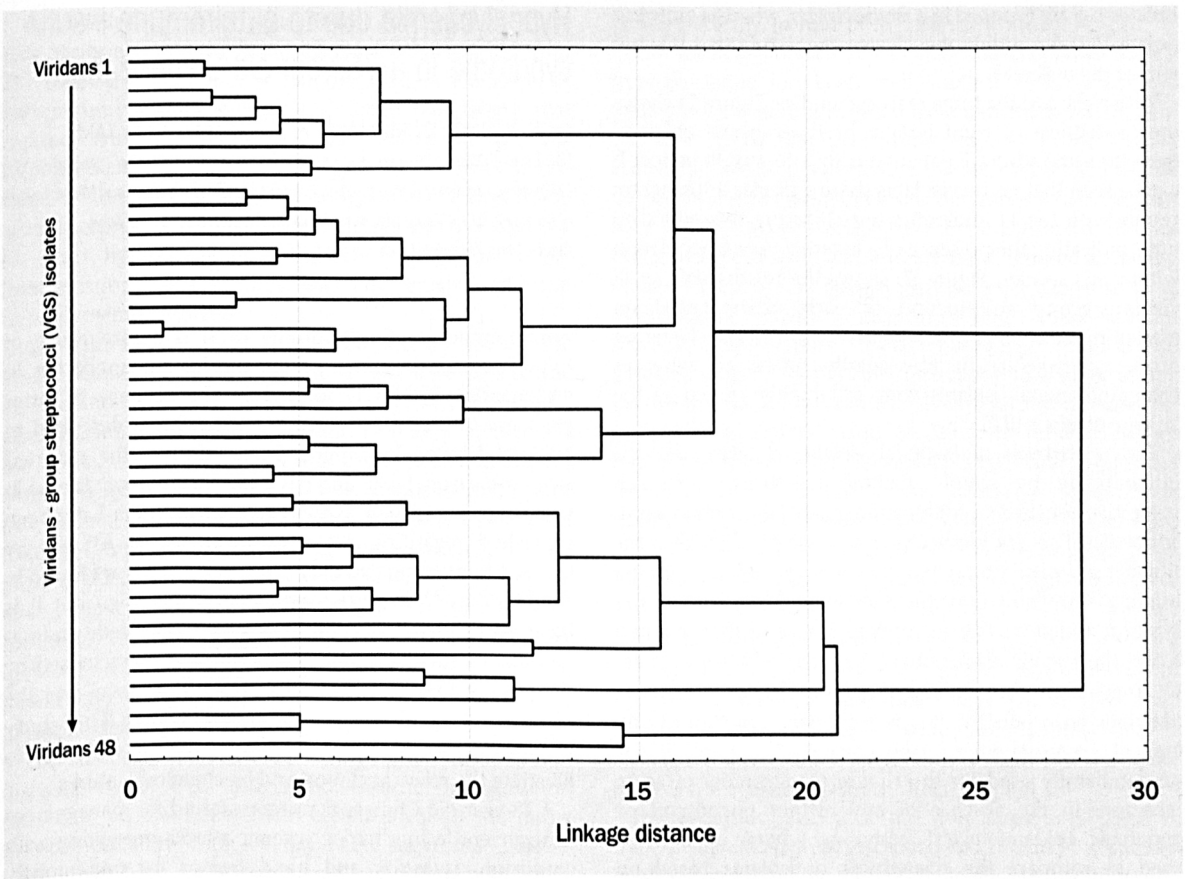

Fig. 2b. Dendogram of isolate relatedness based on complete linkage of Euclidean distances with seven antibiotic agents examined with 48 isolates of viridans-group streptococci.

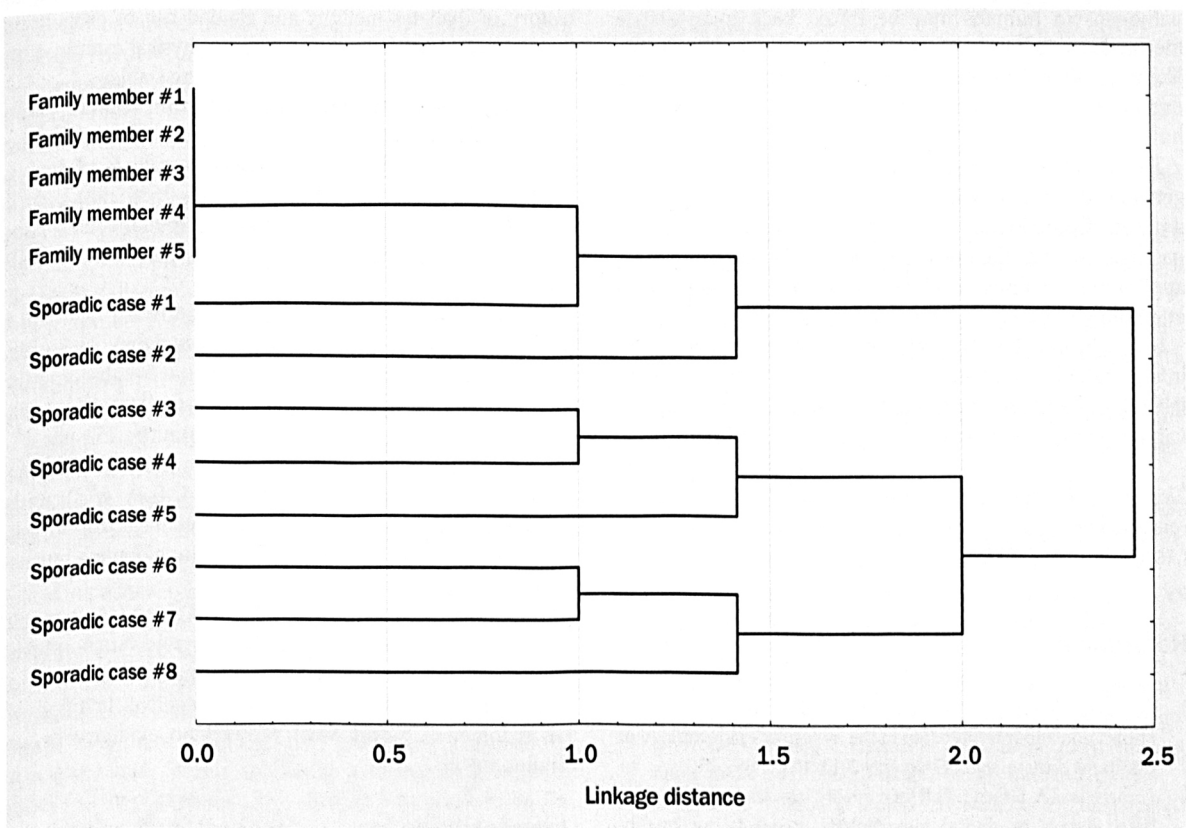

Fig. 2c. Dendogram of isolate relatedness based on complete linkage of Euclidean distances with 12 antibiotic agents examined with a family outbreak of *Salmonella typhimurium*.

isolates 1–9 are grouped in a single cluster, whereas outbreak isolate 10 is not within this cluster, suggesting that it is not part of the outbreak.

With regard to the three real case studies, Figure 2a shows the relatedness of eight isolates of *P. aeruginosa* obtained from the same adult CF patient during a 16-month period. It can be seen that no two isolates share a similar antibiogram profile with the 11 antibiotics tested during this period of time, indicating the presence of a heterogeneous population within this species. Figure 2b shows the relationship of 48 viridans-group streptococci to each other, based on measuring zone sizes (mm). Figure 2c shows the clustering of *S. typhimurium* in five family members, whereas contemporaneous isolates were not highly related at the linkage distance (0.0).

The relatedness of bacterial isolates is often assessed qualitatively by simple mental comparison of each respective organism's antibiogram against a common set of antibiotics. This analysis becomes increasingly difficult as the number of isolates examined increases, as well as when the number of antibiotics examined increases. Many laboratories perform antibiotic disk susceptibility beyond that required to aid therapeutic management, yet relatively few data are obtained from this additional effort. The generation of antibiotic susceptibility data is a primary function of any clinical microbiology laboratory. However, it is predominantly used for the clinical management of acute infection. In the absence of any further phenotypic or genotypic epidemiological subtyping scheme, it is often used to compare the relatedness of isolates based on similarities/differences in antibiotic susceptibility patterns. One limitation to exploiting this is the fact that an inability to perform clustering of antibiotic resistance in clinical pathogens in humans may be traced back to resistance mechanisms in environmental bacteria, and any factors likely to alter (upregulate) resistance in environmental organisms are of potential and eventual consequence to human pathogens.

This small study demonstrated the software's ability to generate dendograms that aid the interpretation of each dataset. Commercial availability of software analysis packages, such as the one examined in this study, allows its application presently in clinical microbiology laboratories attempting to interpret antibiogram information.

In conclusion, this software may be a useful adjunct in the interpretation of antibiogram data in clinical microbiology laboratories and an easy-to-use tool, thereby adding further value to the generation of antibiogram information.

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Hypoglycaemia due to autoimmune insulin syndrome in a 78-year-old Chinese man

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Autoimmune insulin syndrome (AIS) is a rare cause of hypoglycaemia in which a high titre of autoantibodies to endogenous insulin is formed in the absence of prior exposure to exogenous insulin, and was first described in 1973 by Hirata.[1] Epidemiologically, most of the reported cases come from Japan and it is a very uncommon disease in Caucasian population. No epidemiological data have been published regarding the exact incidence of AIS in the Chinese population and only a few case reports are found in the literature.[2–4] The exact aetiology is unknown but it is believed to involve an interplay of genetic predisposition and environmental factors.[5] Clinically, it is characterised by episodes of hypoglycaemia, elevated insulin level and the presence of circulating insulin autoantibodies. This study includes a case report of a Chinese patient with AIS to illustrate the relevant clinical and biochemical features.

A 78-year-old Chinese man was referred for management of recurrent fasting hypoglycaemic attacks presenting with confusion, sweating and hand tremor for one month. His past medical history included hypertension and chronic obstructive airways disease. Medication included prazosin, salbutamol inhaler, terbutaline and theophylline. He had no history of diabetes mellitus and denied use of exogenous insulin or oral hypoglycaemic agents. Physical examination was unremarkable and his vital signs were stable.

During hospitalisation, he was documented to have symptomatic hypoglyacaemia with plasma glucose in the range 1.1–2.1 mmol/L. The simultaneous insulin level during hypoglycaemia was in the range 131–1688 miu/L and C-peptide level was 0.27–0.43 nmol/L. Simultaneous serum growth hormone, cortisol and thyroid function tests were all normal.

Other laboratory studies were significant for mild hypochromic microcytic anaemia (haemoglobin: 11.3 g/dL [reference interval: 13.0–19.0 g/dL), which was subsequently confirmed to be due to underlying β-thalassaemia trait. He had renal impairment (plasma creatinine: 152 μmol/L [85–133 μmol/L]). Liver function was normal. Plasma globulin level was elevated (68 g/L [24–36 g/L]) with albumin:globulin ratio of 0.47. Serum protein electrophoresis revealed a generalised increase in immunoglobulins but no paraprotein band.

Other immune markers (ANA, RF, C3, C4, ANCA) were all negative. Coombs' test was negative. Thyroid function tests were normal and serum antithyroglobulin and antimicrosomal antibodies were both negative. Imaging of the abdomen (CT and MRI) showed no evidence of an insulinoma.

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