

# The biochemical investigation of cases of hypoglycaemia: an assessment of the clinical effectiveness of analytical services

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**Aim:** To assess the extent to which biochemical analytical services contribute to the diagnosis and management of clinical cases of hypoglycaemia.

**Methods:** All cases of confirmed hypoglycaemia, referred during a six month period, were included in the survey. Questionnaires were sent to each referring laboratory requesting information on the clinical progress and current status of the patient.

**Results:** The level of influence exerted by analytical data was assigned in each case and those with similar outcomes combined. Identifiable case groups were: (1) Results not recorded in the patients' notes (15.7%). (2) Inappropriate requesting of insulin and C peptide measurements in cases of diabetes (11.4%). (3) Patient died soon after investigation (20.0%). (4) Patient recovered spontaneously (17.1%). (5) Patient received effective medical or surgical treatment (12.9%). (6) Patient awaiting or not requiring pathology based treatment (31.4%). (7) Inconclusive outcome prompting further investigation (5.7%).

**Conclusions:** Within the timescale of the survey (approximately 12 months), positive progress had been made towards diagnosis and subsequent treatment in only 10% of cases. Another 30% were either awaiting some form of treatment or further diagnostic tests. The remaining 60% did not appear to benefit in any way from the biochemical investigations.

For almost 20 years Supraregional Assay Service (SAS) laboratories have been supporting a system that makes available specialist expertise, both analytical and interpretative, in the investigation of disorders that occur relatively infrequently.<sup>1</sup> The clinical area that has been the focus of attention in the Guildford peptide hormone laboratory is hypoglycaemia.

The variety of referred cases has facilitated the development of an investigative procedure with the objective of providing a potential diagnosis.<sup>2</sup> This process is based on the pattern of biochemical parameters that emerges, but often in the absence of relevant clinical information. As a consequence, the suggested conclusions are often made in isolation from the patient.

The fate of the patient under investigation usually remains unknown unless a positive attempt is made to identify the clinical outcome. Therefore, a survey was undertaken to examine the diagnostic effectiveness of the biochemical investigation and the performance both of the referring laboratory and the Guildford laboratory scrutinised in terms of request management.

## METHODS

### Analytical

Enzyme linked immunosorbent assay kits for the measurement of insulin (Mercodia) and C peptide (IBL) in serum were obtained from Diagenics Ltd (Milton Keynes, UK) and IDS Ltd (Boldon, Tyne and Wear, UK), respectively. The insulin method has a 56% reactivity with proinsulin but does not react significantly with C peptide. The C peptide method has insignificant reactivity with insulin and proinsulin. Both methods are sufficiently sensitive (insulin detection limit, 10 pmol/litre; C peptide, 100 pmol/litre) to measure the low analyte concentrations often encountered in the investigation of hypoglycaemia.

### Survey format

All requests received during the period January to June 1999 and requiring biochemical investigations relevant to the diagnosis of hypoglycaemia were examined. The only criterion for inclusion of a referred case was confirmation of hypoglycaemia in the serum sample received. A questionnaire was sent to each referring hospital laboratory asking for information relevant to the clinical outcome of each case six to 12 months after return of the analytical results. Responses were screened for details on whether:

- (1) The analytical data actually reached patient records.
- (2) The data played any part in patient management.
- (3) Any interpretative advice appearing on the report was appropriate/relevant to the clinical problem.
- (4) The data/advice influenced the progress of any remedial treatment.

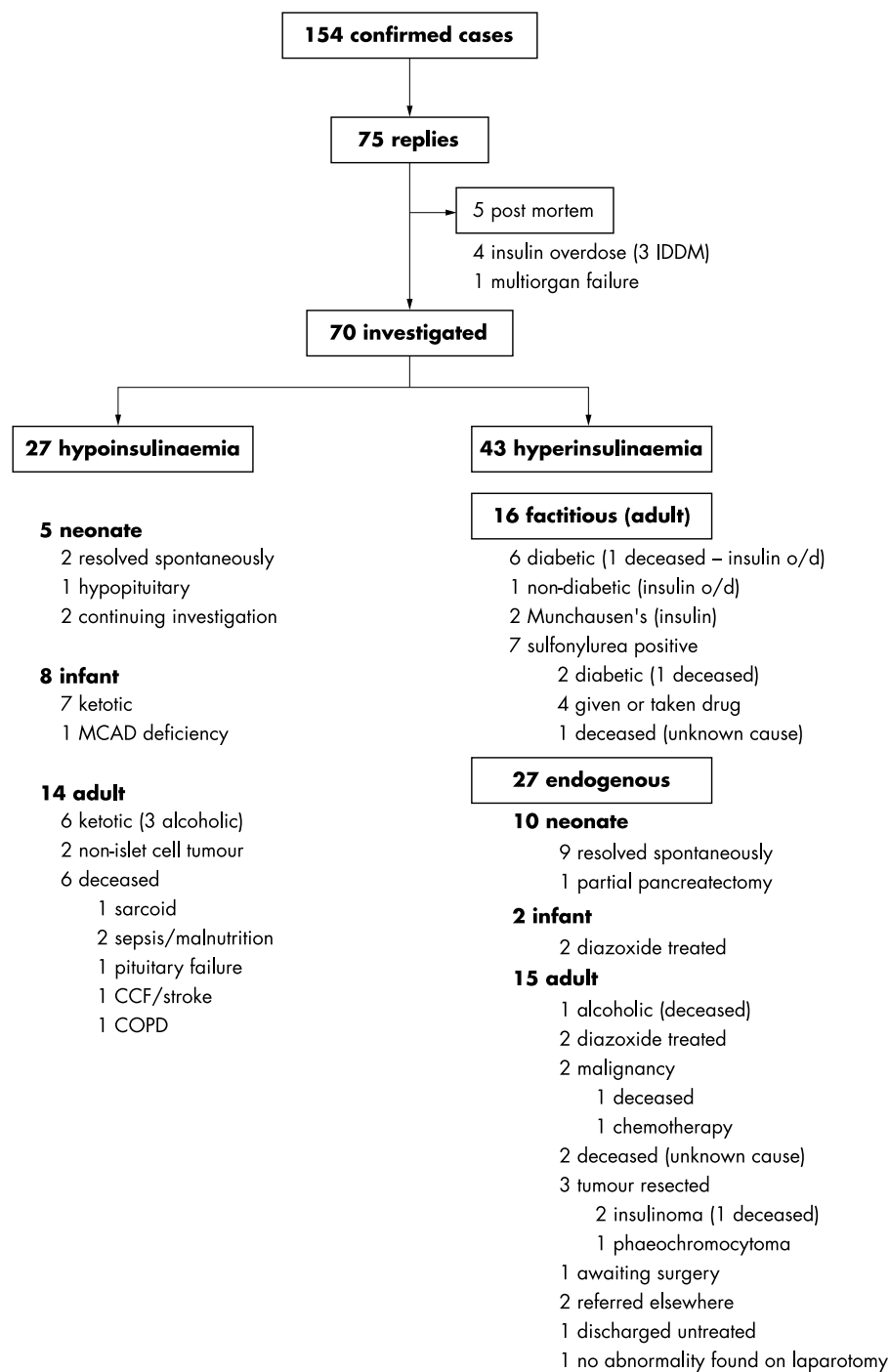
### Classification

Hypoglycaemia was defined as a blood glucose of < 2.5 mmol/litre (less than 3.0 in patients over 60 years old) and where possible was confirmed by analysis of the serum/plasma sample received in our laboratory. Serum insulin > 10 pmol/litre and serum C peptide greater than 100 pmol/litre in the presence of hypoglycaemia was defined as inappropriate hyperinsulinaemia and hyper-C peptidaemia, respectively.

### Age groups

For reasons of clinical interpretation of the biochemical data the patients with hypoglycaemia were divided into neonates

**Abbreviations:** IKHH, idiopathic ketotic hypoinsulinaemic hypoglycaemia; SAS, Supraregional Assay Service



**Figure 1** Cases of hypoglycaemia received January to June 1999. CCF, congestive cardiac failure; COPD, chronic obstructive pulmonary disease; IDDM, insulin dependent diabetes mellitus; MCAD, medium chain acyl-CoA dehydrogenase; o/d, overdose.

(up to 18 days old except one patient of 33 days in whom symptoms had been present since birth), infants (under 3 years), adults (12–92 years), and postmortem cases.

## RESULTS

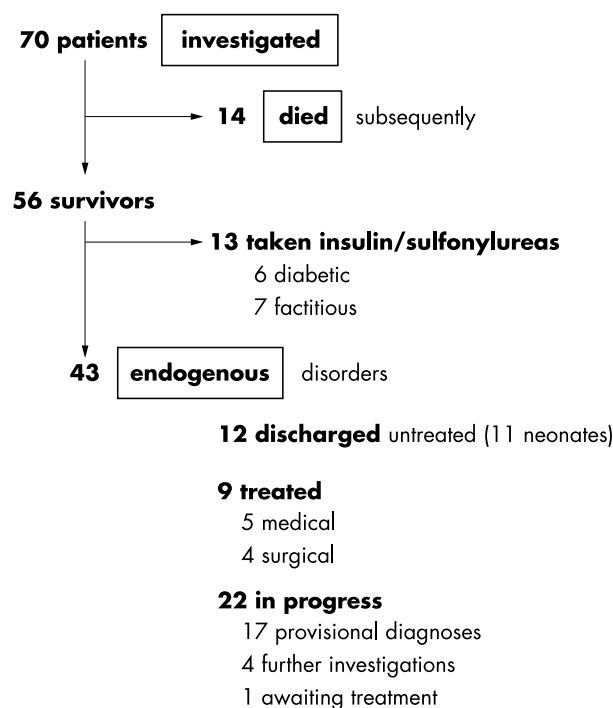
In the six month survey period, 154 cases of hypoglycaemia were confirmed and processed. In 75 (49%) of these further information was supplied subsequently by the referring hospital. Detailed reports and copies of relevant correspondence were supplied by the consultant physician in 23 cases. Copies of discharge summaries were supplied by the consultant biochemist in 17 cases. A summary letter was received from either the physician or biochemist in five and six cases, respectively. Brief notes only were supplied by seven physicians and seven biochemists. In three cases, brief notes were

supplied by the audit office of the requesting hospital. In two cases, the requesting hospital was reluctant to supply clinical information on the grounds that in doing so patient confidentiality would be contravened. In only two of the five postmortem cases was it necessary to request confirmation of the cause of death and this was provided by the coroner's office in both.

Figure 1 summarises the total number of cases examined and the information provided by replies to questionnaires. Figure 2 is a summary of the replies received in terms of clinical outcome.

## Postmortem cases

Postmortem serum samples were referred when there was evidence of hypoglycaemia or when insulin/sulfonylureas



**Figure 2** Fate of patients (within the 12 month survey period).

were in the possession of the deceased. Raised insulin values (between 590 and 17 747 pmol/litre) and suppressed C peptide (< 100 pmol/litre) concentrations were found in four cases, and it was presumed that insulin overdoses were involved. Three of these patients had been under treatment for type 1 diabetes. No explanation was discovered for the fourth. In the fifth case, hypoglycaemia was associated with inappropriately high concentrations of insulin and C peptide in the absence of sulfonylureas. The cause of death was not established but the patient had suffered multiorgan failure, sepsis, and cirrhosis. The five postmortem cases have not been included in the analysis of the rest of this survey.

### Neonatal cases

Ten of the 15 neonatal patients were hyperinsulinaemic. In nine of these, symptoms resolved spontaneously and one patient was subjected to a partial pancreatectomy.

Five neonates, one of whom was born prematurely, had been affected by intrauterine growth retardation. These comprised three who were hyperinsulinaemic and two who were hypoinsulinaemic. No birth information was provided in the other 10 cases.

It was apparent that 11 of the 15 neonates made satisfactory progress towards normal development, whether initially hyperinsulinaemic (nine cases) or hypoinsulinaemic (two cases).

### Infant cases

Inappropriately raised serum insulin was an important factor in only two of 10 cases, and in both cases the patient was treated with diazoxide. In the other eight patients, serum insulin values were appropriately low, being associated with ketonaemia in seven ( $\beta$ -hydroxybutyrate: >600  $\mu$ mol/litre) patients and medium chain acyl-CoA dehydrogenase (MCAD) deficiency in one.

### Adult cases

The 45 adult cases of hypoglycaemia were divided into three groups based on whether insulin concentrations were inappropriately high (hyperinsulinaemia, endogenous or factitious) or appropriately suppressed (hypoinsulinaemia).

In 16 of 31 cases of hyperinsulinaemia, the hypoglycaemia was factitious as a result, in nine patients, of exogenous insulin (high serum insulin, suppressed C peptide) or, in seven patients, sulfonylurea ingestion (serum insulin and C peptide both inappropriately high and sulfonylureas detected in the sample). Survey information revealed that six of the nine cases of insulin administration involved patients with diabetes who were being treated with insulin, one of whom died. In three other cases, patients (not diabetic) had taken insulin, one in unknown circumstances, and two being diagnosed as having Munchausen's syndrome.

In three of the seven positive sulfonylurea cases the test had been specifically requested. Suspicion had been aroused in two cases and involved patients with diabetic spouses. The third patient was identified as having type 2 diabetes. The other four cases were found to be positive as a result of routine screening of all samples with inappropriately high serum insulin and C peptide values. Two of these patients had renal failure and died subsequently. One had been treated for type 2 diabetes but no source of diabetic medication was discovered in the other. The third patient had a diabetic spouse and the fourth had been given the wrong medication.

The remaining 15 cases of hyperinsulinaemia were the result of endogenous hyperinsulinism, potentially caused by autonomous secretion of insulin. Five of these patients died. Of these no cause of death was recorded in two cases, alcohol was a factor in one, unspecified malignancy was present in another, and the fifth had an insulinoma resected but died from unknown causes.

Of the remaining 10 patients, three were undergoing treatment (two with diazoxide, one with chemotherapy), two had islet cell tumours resected, three were awaiting surgery or referral to a specialist centre, one was discharged without treatment, and one underwent pancreatic surgery but no tumour was found.

Suppressed serum insulin and C peptide values were found in 14 patients with a variety of disorders, which in six cases resulted in death. Ketosis was noted in six, and was associated with alcoholism in three. In two cases, the hypoinsulinaemia resulted from the secretion of an abnormal form of insulin-like growth factor II produced by non-islet cell tumours.

### Analytical data

Table 1 lists serum glucose, insulin, and C peptide data in the patient groups. Each group has been subdivided into cases of hypoinsulinaemia or hyperinsulinaemia, which for adult patients have been further classified according to the diagnostic outcome established in fig 1.

All referred samples were analysed for glucose, when adequate volumes permitted. In 39 of the 70 clinical cases examined the documented and checked glucose values were not significantly different ( $p = 0.54$ ) and, on this basis, in a further 19 cases the Guildford results were assumed to be accurate enough for the purposes of our survey. In the remaining 12 cases, glucose concentrations were not measured because of inadequate sample volume, although results were documented in seven of these requests. The glucose data are therefore a combination of results provided by referring laboratories and those produced by measurement after receipt in Guildford.

Only gross differences between diagnostic classifications were observed in the insulin and C peptide data, mainly because of the small numbers of cases and the wide ranges of values in each group.

### Clinical usefulness of the analytical reports

The relevance to the clinical problem to the biochemical tests and any suggested interpretation was assessed from the information supplied. In 11 cases, either the biochemical reports

**Table 1** Mean serum glucose, insulin, and C peptide values in the patient groups according to diagnosis

Patients	Classification	Glucose [n] (mmol/l)	Insulin [n] (pmol/l)	C-peptide [n] (pmol/l)
Neonates	Hypoinsulinaemic	1.9 (0.7) [5] range, 0.9–2.7	24 (5) [3], <10 [2] range, <10–29	107 (5) [2] range, 104–111
	Hyperinsulinaemic	2.0 (0.5) [10] range, 1.3–2.7	185 (206) [10] range, 34–705	1119 (1059) [6] range, 433–3191
Infants	Hypoinsulinaemic	1.7 (0.8) [8] range, 0.5–2.5	21 (8) [2], <10 [6] range, <10–27	243 [1], <100 [4]
	Hyperinsulinaemic	1.9 (0) [2] range, 1.9	281 (274) [2] range, 87–475	– [0]
Adults	Hypoinsulinaemic (ketotic)	1.9 (0.9) [6] range, 0.5–3.0	19 (8) [3], <10 [3] range, <10–26	192 (129) [2], <100 [2] range, <100–284
	Hypoinsulinaemic (deceased)	2.1 (0.6) [6] range, 1.4–2.6	14 (6) [3], <10 [3] range, <10–18	215 (74) [3], <100 [2] range, <100–272
	Hypoinsulinaemic (NICTH)	1.2 (1.1) [2] range, 0.5–2.0	<10 [2]	<100 [2]
	Hyperinsulinaemic (insulin o/d)	1.5 (0.8) [9] range, 0.2–2.6	3028 (4850) [9] range, 124–14100	<100 [9]
	Hyperinsulinaemic (sulphonylurea o/d)	1.4 (0.2) [5] range, 1.1–1.7	215 (148) [5] range, 49–350	2945 (1710) [5] range, 1107–5575
	Hyperinsulinaemic (endogenous)	1.8 (0.5) [15] range, 0.8–2.4	328 (306) [14] range, 47–979	1643 (1013) [15] range, 474–3716

Values are mean (SD).  
o/d, overdose; NICTH, non-islet cell tumour hypoglycaemia.

had been lost (one) or there was no mention of their contents in discharge summaries (10).

Fourteen patients (including two with diabetes) died soon after the biochemical investigations were carried out; therefore, in these cases it was not possible to identify a positive contribution by the biochemical report to the postmortem conclusion as to the cause of the hypoglycaemia.

Of the 45 survivors, six were patients with diabetes for whom insulin and C peptide measurements were not appropriate. In 39 of the original 70 cases investigated, the insulin and C peptide data were adjudged to have made a positive contribution, either directly to a diagnosis or by suggesting further appropriate tests. This group comprised six neonatal patients (five in whom the hyperinsulinaemia was transient and one who underwent surgery) and all 10 infant and 23 adult cases. It is noteworthy that in only three adult patients was pancreatic surgery, indicated by the analytical data, performed within the survey period, an insulinoma being successfully resected in two, one of whom died soon afterwards, and no abnormality being found in the third.

## DISCUSSION

Our study provides an insight into the logistical management of requests by both referring and analytical hospital laboratories. The suggested procedure for the collection of an appropriate sample type and supply of relevant clinical information forms part of the instructions to users of the SAS.<sup>1</sup> The crucial, not to say mandatory, requirement is the establishment of hypoglycaemia in the sample submitted for analysis because it is only under such circumstances that serum insulin and C peptide values can be classified as appropriately suppressed (below assay detection limits) or inappropriately raised (that is, measurable).<sup>2</sup> A previous internal survey of a two year period of requests referred for the investigation of hypoglycaemia revealed that in 31% of the cases hypoglycaemia was not confirmed by the measurement of glucose in the sample submitted for analysis. The diagnostic effectiveness of analytical and advisory reports proved difficult to assess. However, the division of the cases into the three arbitrary age groups did allow some conclusions to be drawn.

Transient neonatal hyperinsulinaemia is a relatively common disorder that resolves spontaneously without intervention other than maintaining an adequate blood glucose concentration.<sup>3,4</sup> Adult hypoglycaemia, on the other hand, presents with a multiplicity of causes,<sup>5</sup> and in many cases a

final diagnosis was not reached within the timescale of the survey (up to one year after the initial presentation). A high proportion of the patients (20%) died during or soon after collection of the blood sample submitted for investigation. In each of these cases, the potential cause of hypoglycaemia was identified (insulin overdose, malnutrition, sepsis, hypopituitarism). Approximately twice as many patients with hypoinsulinaemia died as did those with hyperinsulinaemia, thus confirming the findings of Klatt *et al.*<sup>6</sup>

Patients with hyperinsulinaemia (31) could be divided equally into those in whom the cause was factitious (16)<sup>7</sup> and those in whom there was an underlying pathology (15).

“Adult hypoglycaemia presents with a multiplicity of causes, and in many cases a final diagnosis was not reached within the timescale of the survey”

Many (eight) of the factitious cases were found to involve patients with diabetes who had presumably been inadvertently or deliberately overtreated with insulin or sulphonylureas. A more diligent assessment by the requesting laboratory as to whether further tests were appropriate to the clinical problem could prevent unnecessary investigations and misuse of the SAS. The three patients without diabetes included two who were diagnosed as suffering from Munchausen’s syndrome. As is often the case, they had both attended several other treatment centres and undergone prolonged investigation before the cause of their hypoglycaemia was established.

Sulphonylurea induced hyperinsulinaemia is easily confused with hyperinsulinaemia that has an endogenous cause, usually an insulinoma. Therefore, screening for the presence of sulphonylureas is advisable if unnecessary surgery is to be avoided. Although there was no evidence of deliberate self administration of sulphonylureas in any of the patients in our present survey a recent review in our laboratory has shown that 34% of patients with inappropriately high insulin and C peptide concentrations had sulphonylureas in their blood; in two thirds of whom it was unsuspected. Approximately 20% of such incidents resulted from errors in drug prescribing or accidental ingestion of a relative’s medication and a further 30% were the result of deliberate drug abuse. No drug source or manner of ingestion could be established in some (40%) of the cases and 10% were the result of malicious poisoning (PYP Kwong and JD Teale, 2002, unpublished data).

Pathological disorders involving endogenous hyperinsulinism are usually attributed to abnormal insulin secreting cells,

### Take home messages

- During the 12 months of this survey, positive progress had been made towards diagnosis and subsequent treatment in only 10% of cases
- A further 30% of patients were either awaiting some form of treatment or further diagnostic tests
- The remaining 60% of patients did not appear to benefit in any way from the biochemical investigations
- A more diligent assessment by the requesting laboratory as to whether further tests are appropriate to the clinical problem could prevent unnecessary investigations and misuse of the Supraregional Assay Service

whether benign, malignant<sup>8</sup> or, rarely, ectopic.<sup>9</sup> Owing largely to the difficulty and unreliability of preoperative tumour localisation,<sup>10</sup> many of these cases were not concluded during the survey.

In six of 14 patients with hypoinsulinaemia in our survey a ketotic response had occurred and a diagnosis of idiopathic ketotic hypoinsulinaemic hypoglycaemia (IKHH) had been assigned to them. This unsatisfactory diagnostic label encompasses a large number of diseases, including many inborn errors of metabolism that can, under adverse environmental conditions, produce hypoinsulinaemic hypoglycaemia. Those patients with hypoinsulinaemia who were not diagnosed as suffering from IKHH were suffering from a variety of disorders with poor prognostic outcome, in that five of them died. Two patients were diagnosed as suffering from non-islet tumour hypoglycaemia on the basis of pre-existing malignancy and the typical biochemical profile.<sup>11</sup>

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